CHEMICAL ANALYSIS
OF
FOODS AND FOOD PRODUCTS

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FOODS A

DEDICATED

to

Food, Drug

Chemist,

my wife, MARGARET

rs, ROBERTA LEAH and BERENICE ANITA,

will insure not only them but also all sons

s a surer, better and safer food supply.

D.

NY, INC.

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hout
here.

1945

DEDICATED

to

my wife, MARGARET
and my daughters, ROBERTA LEAH and BERENICE ANITA,
in the hope that it will insure not only them but also all sons and daughters a surer, better and safer food supply.

PREFACE

This book was written with a number of objectives. First and foremost was the desire of the author to give systematic coverage to the salient facts of the chemical analysis of foods and food products and to include certain of the newer aspects of food analysis such as the chemical assay of vitamins, the detection of improper pasteurization of milk, the homogenization of milk, the detection of gums and methods for the detection of newer types of sophistication of foods. Many important analytical procedures have been entirely neglected, not only in the literature, but even by so eminent a society as the Association of Official Agricultural Chemists whose latest edition of methods, 1935-36 contains no word on the detection of the improper pasteurization of milk, nor any chemical methods for the vitamins although Tillman's method for vitamin C has been recognized as adequate since 1928.

The author also has the desire to present a number of topics such as gums, jams and jellies, milk products in other foods, soy bean flour in meats, pumped smoked meats and other timely topics which properly belong in the modern literature of the subject and on which information has heretofore been difficult to obtain.

The author has attempted to present short practical methods which are usable and efficient and are, of course, of vast importance in routine analysis and in general control work. Throughout the book stress is placed on the fact that in all probability the analysis of a food product should fall within known normal limits and that the tendency to adulterate is closely tied to economic factors.

The book is designed for use as an educational text and as a manual for manufacturers for control work. It is hoped that it will prove useful in regulatory laboratories, both governmental and commercial and as a reference particularly in those subjects treated herein which are not treated in other texts in the subject.

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Acknowledgment is made for the abstraction of methods, procedures, tables, etc., of other well known texts. These are noted at times throughout the actual text and at other times in the selected references at the end of each chapter.

MORRIS B. JACOBS

Brooklyn, New York (1938)

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Definitions of Terms and Explanatory Notes

1) The term **water** used in the methods means distilled water.

2) The terms **alcohol** and **ether** refer respectively to 95 per cent ethyl alcohol and ethyl ether. The terms **high grade gasoline** and **petroleum ether** are often used interchangeably.

3) The foods and food products discussed in the text have, if defined, the definitions and standards given in United States Department of Agriculture, Food and Drug Administration, Service and Regulatory Announcements, Food and Drug No. 2, Fifth Revision (1936), and Food and Drug No. 4, Fourth Revision (1937).

4) The following reagents, unless otherwise specified or qualified in the text, have the approximate strength stated and conform in purity with the requirements of the United States Pharmacopoeia.

sulfuric acid	specific gravity 1.84
hydrochloric acid	specific gravity 1.184
nitric acid	specific gravity 1.42
glacial acetic acid	specific gravity 1.048 (25° C.)
hydrobromic acid	specific gravity 1.38
phosphoric acid	85 per cent strength by weight
ammonium hydroxide	specific gravity 0.90

5) All other reagents and test solutions, unless otherwise described in the text, conform to the specifications of the United States Pharmacopoeia or of the American Chemical Society. When the anhydrous salt is intended to be used, it is so stated; otherwise the salt referred to is the crystallized product.

6) In the expressions (1:2), (5:4), etc., used in connection with the name of a reagent, the first numeral indicates the volume of the reagent used, and the second numeral indicates the volume of water. For example, hydrochloric acid (1:2) means a reagent prepared by mixing one volume of hydrochloric acid with two volumes of water. When one of the reagents is a solid the expression means parts by weight, the first numeral representing the solid reagent and the second numeral the water.

7) In making up solutions of definite percentage it is understood that x grams of substance is dissolved in water and made up to 100 cc. Although not theoretically correct, this procedure will not result in any appreciable error in any of the methods given in this book.

8) All calculations are based on the table of international atomic weights, given in the frontispiece.

9) The following abbreviations are used and have the indicated meaning:

g.	gram
cc.	cubic centimeters
l.	liter
C.	centigrade degrees
F.	fahrenheit degrees
ppm.	parts per million
N	normal, with reference to solutions
mm.	millimeters
mg.	milligrams
e.m.f.	electromotive force
R.P.M.	revolutions per minute
lb.	pound
sp. gr.	specific gravity
A.O.A.C.	Methods of the Association of Official Agricultural Chemists

The abbreviations of periodicals, bulletins, circulars, etc., referred to in the footnotes follow the system of *Chemical Abstracts*.

10) As a general rule, the author has given the preparation of the reagent immediately following the naming of the reagent in the method rather than giving a numbered or lettered list, in the beginning of the method. The author has found that in reading methods it is a great disadvantage to read, "and then add 5 cc. of reagent A . . . ." Very often the analyst must look back to the beginning of the method to find out what reagent A is. Only in the case of very complex reagents is the method of preparation detailed in advance.

The proper way to perform a procedure is to read the method at least twice to completion. On the third reading the reagents are prepared. Then if the determination has never been performed by the analyst before, known samples should be used and only after some experience with known samples has been obtained should unknown samples be analyzed.

CHAPTER I

GENERAL METHODS

General Introduction

The student learns early in his educational career, that chemistry is a mathematical science, and that there is no sharp line of demarcation separating the major branches, namely, inorganic, physical, analytical or organic. Thus the fundamental laws are studied and restudied in each branch. The constitution of the atom is treated fully in both inorganic and physical chemistry texts. Identity tests for elements form as important a part of inorganic chemistry as they do in analytical chemistry. Organometallic compounds are treated as exhaustively in some organic texts as in some more advanced books on inorganic chemistry. In a similar manner, there is no sharp differentiation of chemical analysis of food and food products from chemical analysis of other materials. The object in both cases is to recognize the elements or compounds composing the material and to ascertain the percentage composition. Necessarily, methods peculiar to the field of food analysis have been perfected in order to expedite results.

The chemical analysis of food enables us to know the composition of such materials and with the aid of nutritional and biochemical knowledge, to know what we should eat and what we should avoid eating. Accepting the above as a base, food analysis had its beginnings in times immemorial, for we might say, by organoleptic analysis, that is analysis by use of the senses of smell, taste, sight and touch, man learned that certain materials were not fit for food, either because they made one ill or had little food value. In later times, the dietary rules of Christians, Hebrews, Mohammedans and others codified the trial and error analyses of their predecessors. In more recent times, the fact that man ate relatively enormous quantities of food over a period of time without appreciable gain in weight was clearly recognized and became an object of study.

Lavoisier was one of the first to recognize that life is a chemical function, and that food was the fuel of the body. With the demonstration by Wöhler of the conversion of ammonium cyanate, NH_4CNO, an inorganic

substance, to urea, NH_2CONH_2, an organic substance, and the modern methods of organic analysis devised by Liebig, as a consequence of the growth of organic chemistry, the tools needed for the complete development of the science were at hand.

The earliest quantitative analyses of food materials recorded were those of Pearson,[1] in England, in 1795. In these, Pearson estimated the proportions of water, starch, fibrous matter, extractive matters and ash in kidney potatoes. He recognized the presence of fat, acids, and sugar. The earliest European analyses made comparable with those of recent times are perhaps those of milk by Peligot[2] in 1836, those of feeding stuffs by Boussingault[3] in 1836 and 1838, and those of milk by Boussingault and Le Bel[4] in 1839. The earliest American analyses were made by Shephard[5] in 1845 on the ash of rice and those of Salisbury[6] on maize in 1848.

After these earliest investigations the stress was laid on the proportion of carbon and nitrogen in various food materials. Then Liebig and his followers, Playfair, Boeckman and others, during the period from 1840 to 1865, made the first systematic investigations of foods and feeding stuffs by methods more or less similar to those of today. A great advance was made when Henneberg[7] and his associates elaborated the methods for the proximate analysis of foods. A "proximate" analysis is distinguished from an "ultimate" analysis in that it is not a determination for a particular element or compound but is rather an estimation of a certain type of constituent as "volatile matter," "moisture," "fat," "carbohydrate," "ash," "nitrogenous matter," etc. Proximate analyses are more easily made and generally give more useful information.

From these earlier analyses and nutrition investigations grew the belief that a proper diet must consist of a correct amount of protein, fat, carbohydrate, water and ash. These were the substances whose percentage was required for the necessary nutritional interpretation; hence, these were the substances which were determined by analysis. Methods

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1 Atwater and Bryant, U. S. Dept. Agr., Bull. No. 28, revised (1906).

2 Peligot, *Ann. chim. phys.*, [2] 62, 432 (1836).

3 Boussingault, *Ann. chim. phys.*, [2] 67, 225 (1838).

4 Boussingault and Le Bel, *Ann. chim. phys.*, [2] 71, 65 (1839).

5 Shephard, *Am. Quart. J. Agr. Sci.* 1, 122 (1845).

6 Salisbury, Trans. New York State Agr. Soc., p. 678 (1848).

7 Atwater and Bryant, U. S. Dept. Agr., Bull. No. 28, revised (1906).

for the analysis of these constituents form a preponderant portion of food analytical literature.

In still more recent times, the realization that the diet problem was not solved merely by adequate utilization of a proper portion of protein, fat and carbohydrate, the three great foodstuffs, lead to the successful search for other substances necessary for the maintenance of health and life. We now know that vitamins, hormones, minute amounts of certain metals, iodine, and other substances and elements are necessary. With this knowledge has grown the development of methods and procedures for the determination and estimation of minute quantities of these substances, so that the science of food analysis is as replete with inorganic, as with organic, qualitative and quantitative methods.

The growth of food analysis as a science is based on four main factors:

1) The desire to obtain nutritional and biochemical knowledge, that is, the knowledge necessary to provide for the well-being of the living organism from the food point of view.

2) The standardization of production and manufacture of food products by means of control analyses as a commercial development.

3) The tendency for governmental regulation in order to protect its citizens from deleterious foods and from being defrauded by adulterated and sophisticated foods.

4) The use by the government of analysis as a base for revenue, by taxation of foods and beverages.

The latter two factors have given a governmental and therefore regulatory cast to the science of food analysis from earliest times in contradistinction to other analytical sciences. In the United States, the Federal Government passed the Wiley food act on June 30, 1906. The function of this law was to control the interstate commerce of adulterated, deleterious and misbranded foods and drugs. It was termed an act preventing the manufacture, sale or transportation of adulterated or misbranded or poisonous or deleterious foods, drugs, medicines and liquors.

Standards, both mandatory and discretionary, for food products, have been set by governmental agencies for the protection of food manufacturers, dealers, and consumers. However, minimum standards have one drawback. Some manufacturers and producers have a tendency to keep their products just at, or slightly above, the minimum standard, thus actually causing a lowering of the average product. For example,

vinegar produced under normal fermentation conditions has an acidity varying between 4.5 to 5.5 per cent. A minimum was set by the Department of Agriculture of the United States of 4 per cent. This minimum was an outside lower limit. The result has been that most of the vinegar sold is now diluted down to the quoted minimum acidity.

Food analysis is then, in the main, a branch of analytical chemistry. It has need of both qualitative and quantitative analysis. Its interest lies in determining not only what, but also how much, of a component may be present in a food. Such measurements enable one to decide whether a foodstuff is adulterated or unadulterated.

Error and Accuracy

The degree of accuracy desired in food analysis is conditioned by a number of factors not ordinarily considered in other analytical fields. Thus analytical results for nutritional and biochemical investigational work need be more accurate than those of control work, where the primary purpose is to determine whether or not the food product falls within required limits. A further factor concerns the legal aspect of much food analytical work. Here, too, great care must be taken.

Nevertheless, in the measurement of these analytical quantities, the beginner and often the regular analyst is prone to make the mistake of being too accurate. Every measurement entails some error, and to work far outside the limits of that error is to involve useless labor with no gain in accuracy. The accuracy of the final result is quantitatively governed by the accuracy of the least accurate measurement. Thus if we are performing an analysis in which an instrument is used that can be read to only 1 part per thousand, there is waste of time and effort in weighing the original material to be analyzed with an accuracy much greater than 1 part per thousand.

Let us assume that we are analyzing butter for moisture. We have the use of an analytical balance that is accurate to 0.1 mg.

The dish weighs	15.6028 g.
butter weighs	3.0006
combined weight	18.6034
weight after heating	18.1553
moisture	0.4481
per cent moisture	14.94%

Suppose instead of weighing to 0.1 mg., we weigh to the nearest mg.

The dish weighs	15.603 g.
butter weighs	3.001
combined weight	18.604
weight after heating	18.155
moisture	0.449
per cent moisture	14.9%

It is obvious that the accuracy desired is obtained by weighing to the nearest mg. for whether the butter contains 14.94 per cent or 14.9 per cent is not of great moment in food analytical chemistry. Indeed, it has been demonstrated that the error involved in the sampling of the butter is greater.

Of course, if we had to make an analysis that required accuracy to 1 part in a thousand, we would have to weigh to at least 0.1 mg. for less than a 1 gram sample, in order to obtain that precision. On the other hand, if we were weighing a substance that lost or gained moisture rapidly, the additional time needed to give an exact weight would be greatly overbalanced by the error involved in the respective gain or loss of moisture.

If, instead of using arithmetical means for calculating, we were to use a 10 inch slide rule our accuracy would be reduced to 1 part in 500 or 1 part in 800, for we could not read the rule closer than that. Very often accuracy with 1 per cent, 10 per cent, and even 100 per cent error is not at all flagrant. Thus, if a foodstuff contains 0.1 ppm. of lead or 0.05 ppm. is not of material difference in most cases. If 0.05 ppm. is the correct result, then 0.1 ppm. is 100 per cent in error.

We should be as accurate as possible but not so much so that we become impractical. The point involved and to be stressed is that accuracy is indissolubly linked with practicality in food analysis.

Throughout the text more than one procedure is often detailed for any particular determination. This is done intentionally, for the analyst is wont to find one method preferable to another. Furthermore, in the opinion of the author, a check result obtained by different methods is more indicative of the true estimation than a check result obtained by the same method.

Sampling

A most important matter to be considered by the food analyst, although not directly his province, is the proper sampling of the food or

food product to be analyzed. There are probably as many incorrect determinations made because a sample was improperly taken as because of the combined errors of preparation of sample, manipulation, calculation of result, etc. The failure to obtain a proper sample makes a subsequent analysis worthless.

Drawing a proper sample from small containers, packages or batches, such as are customarily sold at retail, is relatively easy for, if small enough, the entire batch or package may be taken as the sample. Since these are usually manufactured or processed foodstuffs, they may be taken as representative.

Moderately large batches present greater difficulties. If the product is a liquid or a powder, it should be mixed thoroughly by stirring or by pouring from container to container, if possible, and then an adequate portion withdrawn for analysis. If the product is a solid, such as butter or margarine, the withdrawal of sections by means of a trier is probably the best procedure. If the product is frozen, such as frozen eggs, sections may be withdrawn by use of a drill.

Large batches, lots or loads of food, such as grain, nuts, fruits, etc., are very difficult to sample properly. It is impracticable, for example, to mix carload lots or shiploads. Hence, when large lots are being sampled, portions of the material are taken from various sections. These portions are mixed thoroughly and from the mixture a suitable sample is drawn. In the case of foods stored in boxes, crates, bags or large cans, portions taken from a representative number of containers are mixed and from the composite a sample is withdrawn for analysis. At times, especially if it is difficult to obtain a representative composite, it is preferable to obtain a number of samples from a large lot rather than to attempt to get one composite representative sample.

Sampling Instruments.—Various implements have been devised to assist in proper sampling. The more important of these are the "thief," sampling tube and trier. The thief, which is used for sampling liquids, is a long tube, about 2 to 3 ft. in length, which has holes in a cap at the bottom end. The tube is inserted in the product to be sampled and when the liquid has risen to the same level as the surrounding liquid, the tube is closed by pushing the cap against the bottom of the container. The thief is then withdrawn and the sample transferred to the sample bottle. An alternative type of thief is the oil thief, Fig. 1, used for sampling oil in drums. It is a copper tube, about 3 ft. long and 1¼ in. in diameter,

with cone shaped ends having an opening, ⅜ in. in diameter. Three legs are placed on the lower end to hold the opening ⅛ in. from the bottom of the drum. Two rings, soldered to opposite sides, at the upper end, permit holding the thief with two fingers leaving the thumb free to close the upper opening and thus withdraw the sample.

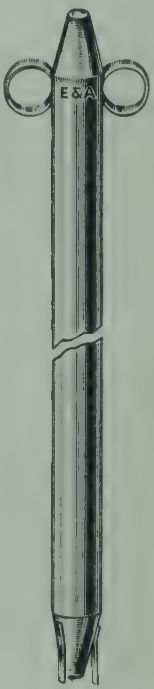


FIG. 1. Oil Thief

(Courtesy of Eimer & Amend)

The sampling tube is generally a brass tube 2 to 3 ft. long and ½ in. to 1 in. wide, with a conical sharp tip at one end, and a handle at the other. A slot extends the length of the tube almost from the tip to the handle. The tube is used mainly for sampling powders, such as milk powder, and coarser materials such as grains. The tube is introduced into the container, which may be a bag, with the slot on the under side. It is then turned so the tube is filled and a core of the material can be withdrawn. Another type, Fig. 2, for securing samples representative of different levels in a container, consists of two brass telescopic tubes with registering slots opened or closed by rotation of the inner tube, the outer tube being provided with a sharp point to facilitate penetration into the sample to the full depth of the tube.

A trier is in reality a very long gouge. It is about 2 to 3 ft. long and ¾ to 1 in. wide. The tip and sides are sharpened so that after insertion into the food material to be sampled, say butter or margarine, rotation of the trier will cut a core of foodstuff that can be removed and transferred to the sample jar or bottle.

To use the trier or sampling tube properly, the instrument should be inserted practically its full length from a point near a top edge or

Trier

FIG. 2. Sampling Tube. (Courtesy of Eimer & Amend)

corner through the center to a point diagonally opposite the point of entry. Usually 2 more triersful are taken from points equidistant from the first.

Procedure of Sampling—The number of cases, cans, tubs, bags, boxes and other containers of a lot to sample to obtain a representative sample is not a simple matter. For some foodstuffs the proper procedure has been studied, for others no set rule has been established.

In the case of single packages or a small batch, as was previously stated, if it be convenient, the entire package or batch should constitute the sample. For moderately larger batches or lots, from 10 to 20 per cent of the number of packages comprising the batch or from 5 to 10 per cent of the weight of the food material should be sampled and sufficient should be taken to yield from 1 to 2 lbs. of sample. For very large lots such as case lots, bag lots, churn lots, etc., a general rule to follow is to sample a number of containers equivalent to the square root of the number in the lot. If experience has shown that no composite sample can be made, the food taken from each container sampled should be analyzed separately.

A method in common usage for obtaining representative samples is the procedure of quartering. Combine the portions obtained from various sections of the lot and after mixing as thoroughly as possible by rolling in a sheet or blanket, if the sample is large, or paper, if the sample is of moderate size, form the material into a cone. Flatten the cone into a circular shape and divide into quarters. Take two opposite quarters, that is, quadrants 1 and 3, and repeat the above process. However, after dividing into quarters this time, the opposite quarters to those used before, namely, quadrants 2 and 4, are taken. This process is continued until a sample small enough for submission for analysis is secured. If permissible, the material is ground and then reground to a finer mesh before each quartering. From 1 to 5 lbs., depending on the amount of material comprising the lot and the amount needed for analysis, should constitute the size of the sample.

The foregoing are minimum sampling requirements and in case of doubt, the food material should be resampled, especially if analysis shows that commercial agreements or sanitary regulations are being violated.

There are further legal factors regarding the question of proper sampling. In some communities the regulatory codes governing foods require that the food material sold to the purchaser be within the standards set by the code. In these instances the purchased article constitutes the representative sample. Another factor concerns the matter of leaving a portion of the sample taken with the owner of the foodstuff for check analysis by his own chemist. This is a fair precaution.

not only for regulatory agencies but also in commercial relationships. If the material being sampled is small canned or bottled goods, other cans or bottles of the same lot number are usually sufficient for the duplicate sample.

All food materials sampled and not in original sealed containers that are likely to lose moisture or otherwise undergo change should be placed in hermetically sealed containers, if practicable, by the person taking the sample. The sample, so bottled or jarred, should be sealed as a precaution against tampering before receipt by the analyst.

Net Contents

In general, we are interested not only in the food and the food product itself, but also in its container and label. This interest arises because some packaged articles are either short-weight or short-volume and often are mislabeled and misbranded. Consequently, the food analyst almost invariably determines the net contents, the net weight and net volume. This may be done in a number of ways. If a large number of empty and filled containers are available, weigh the empty containers and determine the average weight. Repeat this process with the filled containers and obtain the average weight of a filled container, then the difference between the weights of the average filled container and the average empty container equals the average net weight of the contents. To determine the volume of the container, fill the empty containers with water at a definite temperature and weigh. The average weight of the water divided by the weight of 1 cc. of water at that temperature is equal to the volume of the container. It is assumed that all the weights are in grams. If the material is a liquid, the net volume is determined by dividing the net weight in grams by the specific gravity of the material.

The general procedure for a container of glass or tinned or other material that may be washed and dried is the following. Weigh the container and the contents as received. Remove the contents, wash and dry the container. Weigh. The difference in the weights is the net weight of the contents. Fill the container with water at a definite temperature and weigh. The weight of the water divided by the weight of 1 cc. of water at that temperature is equivalent to the volume of the container. If the contents of the container is a liquid, the net weight divided by the specific gravity yields the net volume.

If the food product is a paper packaged frozen or solid article, such

as ice cream or cheese, weigh the contents in the frozen or solid state. Measure the dimensions of the wrapper with an ordinary or micrometer rule. Remove the wrapper. Clean, dry and weigh. The gross weight less the weight of the wrapper equals the net weight of the contents. In the case of a frozen product, the difference in the temperature of the material being weighed and the surroundings introduces an error. The net weight divided by the volume calculated from the measured dimensions of the container yields the approximate density of the material. This method can be used for any size or shape container such as a cone or frustrum of a pyramid. Thus if the package has the shape of a frustrum of a pyramid

$$V = \frac{h}{3}[a + a_1 + \sqrt{aa_1}]$$

where

$V =$ volume
$h =$ height
$a =$ area of top
$a_1 =$ area of bottom.

Similar formulas may be obtained for other shapes in many reference texts.

For some types of packaged products, it is necessary to obtain the drained net weight as in the case of canned oysters, or the net weight free of brine as in the case of anchovies or other fish. To obtain the drained net weight, open the container and transfer the contents to a skimmer or sieve, after allowing to drain for a stipulated time, weigh the contents. This weight is the drained net weight. To obtain the net weight of salted fish, remove the fish from the container and free the fish as well as possible from the salt and weigh the fish. This weight is the net weight of the fish.

In calculating net volume from net weight, weights being in avoirdupois ounces and volume in fluid ounces, the following formula should be used.

$$V = \frac{W}{D_{20}^{20}} \times 0.961$$

where

$V =$ net volume in fluid ounces at 20° C.
$W =$ net weight in avoirdupois ounces.
$D_{20}^{20} =$ specific gravity of the liquid at 20° C. referred to water at 20° C.

Particular attention should be paid to the label of packaged articles for misleading and misbranded statements.

Preparation of Sample

In order to be reasonably certain that the results obtained at the end of an analysis are correct, the sample to be analyzed must be properly prepared. This preparation consists in insuring homogeneity of the sample so that any portion subsequently taken for analysis will represent the whole foodstuff. In general, such homogeneity is obtained in samples that are liquid, pasty or emulsified by shaking the sample and stirring it thoroughly or by transfer from vessel to vessel immediately prior to removing a portion for analysis. Care should be taken not to incorporate air in case specific gravity determinations are to be made. Solid samples should be ground and reground at least three times or comminuted by suitable means. For this purpose, a meat grinder, coffee mill, power grinder, colloid mill, mortar and pestle and knives may be used, in accordance with the particular food product being prepared. Samples that are likely to lose moisture should be stored in mason jars or other rubber sealed or hermetically sealed containers. Samples that deteriorate easily and that must be kept for a long time should have a preservative added, after analysis for preservatives shows their absence. Samples that are likely to spoil should be kept in a refrigerator. Other samples should be dried, ground and stored in a suitable container.

Determination of Specific Gravity

Westphal Balance—In food analysis, specific gravity determinations are generally performed only on liquid foodstuffs. Specific gravity may be measured in a number of ways. A simple and convenient method, where sufficient sample is obtainable, is the use of the Westphal balance. This instrument is illustrated in Fig. 3. It is based upon the principle that a body immersed in a fluid is buoyed up by a force equal to the weight of the liquid displaced. The balance consists of a stand with a leveling screw supporting a beam balanced on a knife edge. The beam has a plummet suspended from one end and counterpoised by a fixed weight with a pointer at the other end. The plummet is made of glass, contains a thermometer and is weighted at the bottom. It is constructed so that it will displace exactly 5 g. of water at 15.5° C. The distance on the beam between the knife edge and the point of support for the sinker

is divided into ten equal parts. The balance is equipped with 5 horseshoe weights or riders. Two are large and of equal weight, namely 5 g., so that one of them placed at the end from which the plummet is suspended, will just counteract a buoyancy of 5 g. The other three weights are respectively one-tenth the weight of the next greater weight. These weights when placed on the notches, that is the points of equal division

FIG. 3. Westphal Balance

of the scale, read respectively, unit, tenths, hundredths, thousandths, and ten thousandths.

To measure the specific gravity, after the beam is balanced in air by adjusting the set screw, the liquid is cooled to several degrees below 15.5° C. and is placed in the cylinder and the plummet is immersed in the liquid. Care should be taken to see that no air bubbles adhere to the sinker. The weights are placed in order, until the beam is balanced. For example, if for a substance whose specific gravity is greater than water the first weight is placed on the hook, the second at notch 1, the third at notch 5, the fourth at notch 1 and the last at notch 6, then the specific

gravity of this liquid is 1.1516. For substances lighter than water the first large weight is not used. If for such a substance as shown in the illustration the second weight is placed at notch 9, the third placed at notch 1, the fourth at notch 5 and the fifth at notch 5, then the specific gravity of this liquid is 0.9155. If two riders have to occupy the same position on the beam, the lighter weight is customarily suspended from the larger weight. There are other types of apparatus based on the

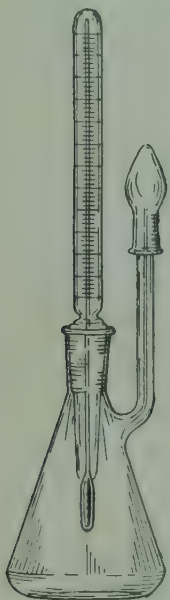


FIG. 4. Specific Gravity Bottle

(From Thurston's Pharmaceutical and Food Analysis. D. Van Nostrand Co., Inc.)

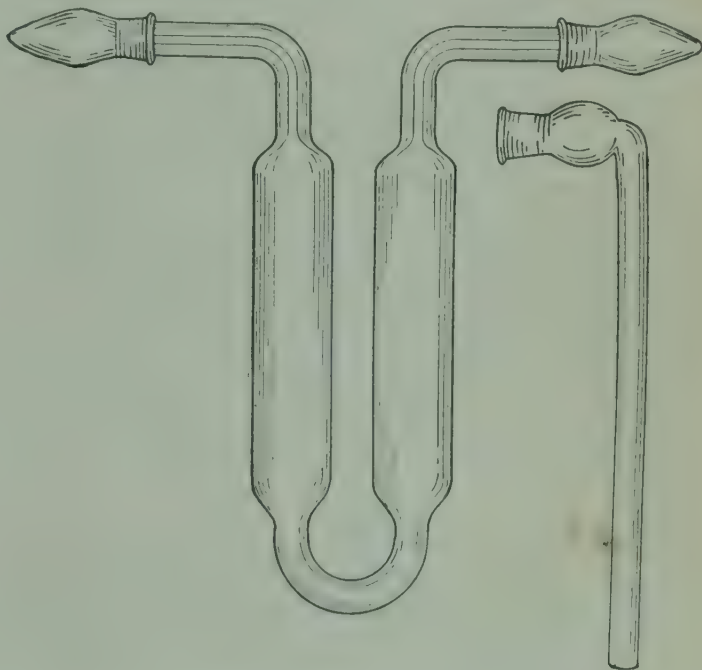


FIG. 5. Sprengel-Ostwald Tube

(From Thurston's Pharmaceutical and Food Analysis. D. Van Nostrand Co., Inc.)

same principle. Readings should be made at 15.5° C. or at the temperature for which the plummet is calibrated.

Pyknometer—A pyknometer is a small, light flask, usually made of glass, of definite volume. It can be used, therefore, to weigh equal volumes of liquids at a given fixed temperature. The ratio of the weights of these equal volumes yields the specific gravity or if using the metric system, the density. The common forms are the specific gravity bottle, Fig. 4, and the Sprengel-Ostwald tube, Fig. 5. The pyknometer

method is a very accurate means of determining specific gravity. There are various forms of specific gravity bottles. Some are equipped with a capillary side arm and thermometer, as in the illustration. Others consist of a small flask fitted with a ground stopper having a capillary.

The weight of the pyknometer must be obtained, when it is clean, empty and dry. It is filled carefully so that no air is incorporated with the liquid cooled to several degrees below the temperature at which it will be weighed. The pyknometer and its contents are allowed to come to temperature either by allowing to stand or by placing in a bath. It is then wiped dry. The excess liquid is removed by means of blotting or filter paper. In the case of the Sprengel-Ostwald tube, the volume is adjusted to the mark by this means. In the case of the weighing bottle, the capillary tube is carefully wiped and the cap placed on. The pyknometer is then weighed. This is generally done at 20° C. The weight of the liquid, divided by the weight of the same volume of water, determined by calibrating the pyknometer by weighing it dry and filled with boiled, cooled water, is the specific gravity. If the specific gravity of the liquid is to be referred to water at the same temperature as that at which the measurements were made, then the formula

$$D_{t^\circ}^{t^\circ} = \frac{W_1}{W}$$

where

D = specific gravity
t° = temperature designated, usually 20° C.
W_1 = weight of a volume of liquid
W = weight of an equal volume of water

is used.

In order to refer this specific gravity to water at its maximum density 4° C., the following formula is used.

$$D_{4^\circ}^{t^\circ} = \frac{W_1}{W} \times D_{W_{t^\circ}}$$

where

$D_{4^\circ}^{t^\circ}$ = density of liquid referred to water at 4° C.
W_1 = weight of volume of liquid
W = weight of an equal volume of water.
$D_{W_{t^\circ}}$ = density of water at temperature t°.

Hydrometer—Another but much less accurate means of determining specific gravity is the use of a specific gravity spindle or hydrometer, Fig. 6. The spindle is a float usually cylindrical in shape with a bulb in the middle, a slender upper stem and weighted bottom. The stem is graduated with a scale to read specific gravities directly or some arbitrary scale such as Brix, Twaddell, Baumé, Quevenne or New York Board of Health. The lactometer, alcoholometer, saccharometer and Baumé spindle are types of specific gravity spindles.

The liquid is placed in a suitable cylinder at the definite temperature for which the spindle is calibrated and the hydrometer is immersed in the liquid. The hydrometer is pushed slightly below the equilibrium point and is allowed to rise to its proper level. This is done to overcome surface tension and viscosity effects. The specific gravity is read directly or the degrees of the arbitrary scale are read and then the density obtained from tables.

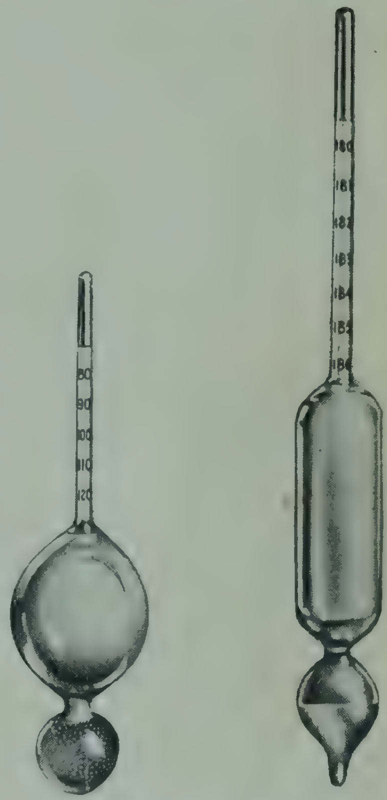


FIG. 6. Left—Lactometer, N. Y. Board of Health
Right—Hydrometer

A convenient method of using hydrometers is to use a set consisting of two range finding hydrometers, one for liquids lighter than water and the other for liquids heavier than water, and nineteen others more finely graduated. These nineteen are graduated from specific gravity 0.700 to specific gravity 1.850, each hydrometer measuring an interval of 0.060 except the last which measures an interval of 0.070. One of the two range finding hydrometers is used first and from the reading the appropriate finely graduated hydrometer is selected and subsequently used.

Flotation Method—This method is based on the principle that a solid will just float in a liquid of the same specific gravity. The method is often used for waxes and hard fats. A series of suitable liquids of graded specific gravities must be made, e.g. water diluted with varying proportions of alcohol. That mixture of definite specific gravity in which

a small smooth piece of substance free from air just floats is the specific gravity of the substance.

Specific Gravity Centrifuge Bottle[8]—This method is of great value in determining the specific gravity of materials of high viscosity which retain air bubbles. This may be done by centrifuging the materials in weighed flasks. The specific gravity of tomato products such as pulp, purée, paste, catsup and chili sauce may be conveniently estimated by this method.

Obtain the weight of an empty, clean, dry flask of about 125 cc. capacity that is of such shape as to fit a Babcock centrifuge holder. A Babcock flask, Fig. 39, with its neck removed, though only half the volume, serves well. Fill to overflowing with water at a temperature slightly below 20° C. and allow the temperature of the water to rise slowly to exactly 20° C., having a thermometer in the water in the flask. Remove the thermometer, wipe the flask dry on the outside and add water drop by drop until the flask is exactly level full, or slightly overfill the flask and remove the excess water by passing a microscope slide over the opening. Weigh at once to the nearest 0.01 g. Subtract the weight of the empty flask to obtain the weight of water this flask holds at 20° C.

Cool the sample to 16–18° C. Fill the flask with the sample and place it in the centrifuge with a suitable counterpoise. Whirl the centrifuge cup for from one-half to one minute at a speed of about 1000 R.P.M. The air bubbles are removed by the whirling and the surface of the pulp may now be considerably below the top of the flask. Fill the flask to the top and whirl in the centrifuge again. Remove the flask from the centrifuge, wash the outside, wipe dry, then insert a thermometer. When the temperature is just 20° C., remove it and add a few more drops of pulp or sample so that the level of the product comes exactly even with the top of the flask or slightly overfill and strike off with a straight edge. Weigh the flask and contents, at once, to the nearest 0.01 g. Calculate the apparent specific gravity by dividing the weight of sample in the flask by the weight of water at 20° C. that the flask was found to hold.

Determination of Moisture

Moisture is the material lost by a foodstuff on heating not much higher than the temperature of boiling water or by allowing to stand

[8] Bigelow, Smith and Greenleaf, Nat. Canners' Assn. Bull. 27-L (1924).

over a dehydrating agent or by heating in a vacuum. It is generally considered to be water but actually is the total volatile matter at this temperature.) When water content alone is to be determined, direct heating methods are not applicable. The residue remaining after using the direct heating, vacuum oven, or over sulfuric acid methods, is termed total solids.

Direct Heating—The estimation of moisture is one of the most often performed determinations in food analysis. Usually, this is done by weighing a definite amount of material into a low, flat-bottomed, dried and tared dish, to which, sometimes, dried and weighed sand or asbestos has been added. The dish and its contents is placed in a water oven, or air oven thermostatically controlled at 100° C. to 105° C., or some similar oven for a stipulated time, or until successive weighings show no further loss. At the end of this time, the dish is removed from the oven, placed in a desiccator, allowed to cool and weighed. The loss in weight equals the moisture. The loss in weight divided by the weight of the original sample is the per cent moisture. The sand and asbestos aid the drying process probably by providing focal points for evaporation and by decreasing the possibility of superheating.

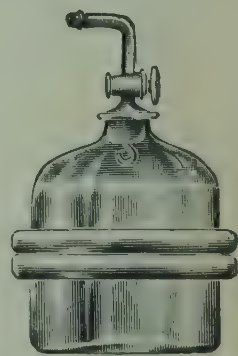


FIG. 7. Vacuum Desiccator

(Courtesy of Eimer & Amend)

Vacuum Oven—Some foodstuffs contain substances, for example d-fructose, levulose, that are decomposed by heating at 100° C. under atmospheric pressure. Such materials should be dried in a vacuum oven under reduced pressure at 70° C. until successive weighings show no further loss in weight.

Over Sulfuric Acid—Drying without heat over sulfuric acid is slow and tedious, but nevertheless should be used where very exact determinations are necessary. Weigh into a flat-bottomed, tared, metal dish, fitted with a tight cover, 2–4 g. of material. Place the dish, uncovered, in a vacuum desiccator, Fig. 7, containing 200 cc. of fresh sulfuric acid. Exhaust the desiccator by means of a vacuum pump. Turn the desiccator around four or five times during the first 12 hours. After

24 hours open the desiccator, causing the incoming air to bubble through the sulfuric acid. The cover is then replaced and the dish weighed. Repeat, using fresh sulfuric acid until the weight is practically constant. The percentage moisture is calculated as directed above.

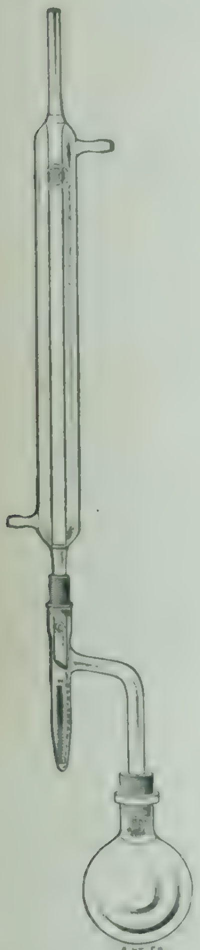


FIG. 8. Immiscible Solvent Apparatus

(Courtesy of Arthur H. Thomas)

Immiscible Solvent Distillation Method.—A rapid and fairly accurate method for determining relatively small quantities of moisture is the immiscible solvent distillation method. This method is of especial importance, when it is desired to distinguish between water and volatile matter, as for example in spices, which contain volatile oils.

Weigh into a 250 cc. flask or transfer to this flask sufficient sample to yield 2–5 cc. of water. Cover the material with about 75 cc. of toluene. In case of syrups add 10 g. Filter-Cel before addition of syrup and toluene to the flask and another 10 g. Filter-Cel just prior to distillation. Other solvents immiscible with water may be used in place of toluene, as for example xylol. Tate and Warren [9] recommend heptane. Attach the flask, Fig. 8, with a Liebig condenser in a reflux position by means of a Bidwell and Stirling receiver, Fig. 9. Fill the receiving tube with toluene by pouring the liquid down the condenser. Distill slowly at the rate of about 2 drops per second and then more rapidly when practically all of the water is over. Just prior to ending the distillation, wash down the condenser with toluene by pouring some through the top. Continue distilling and if no more water comes over, the determination is finished. If any water adheres to the side of the condenser or tube, rub it down by means of a rubber band attached to copper wire, while washing down with toluene. Now distill for a short time longer. No water should adhere if the apparatus has been thoroughly and properly cleaned with cleaning solution and then washed and dried.

[9] Tate and Warren, *Analyst* 61, 367 (1936).

Read the volume of the water in the distillation tube and calculate the percentage moisture assuming that the water volume is equivalent to its weight in grams, by dividing by the weight of the original sample taken and multiplying by 100.

As was previously stated, the direct heating method does not determine the true water content. It estimates the total volatile matter at 100° C. When it is desired to distinguish between volatile matter or apparent moisture and water content, some other method such as the immiscible solvent distillation method must be used.

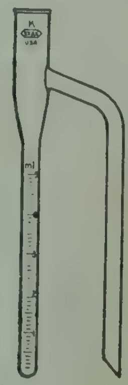
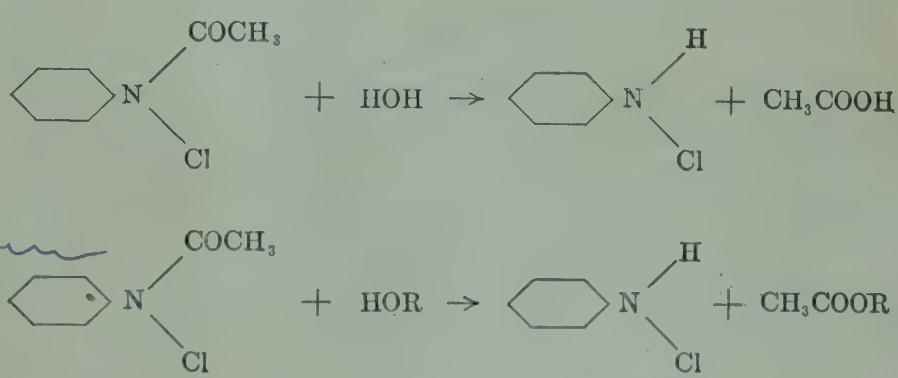


FIG. 9. Bidwell-Stirling Receiver

(Courtesy of Kimball Glass)

Smith and Bryant[10] Method—A rapid quantitative procedure for determining varying amounts of water in organic liquids utilizes the fact that acetyl chloride in the presence of pyridine produces two moles of titratable acid with water, whereas reacting with an alcohol only one mole of available acid is produced. Fatty acids can be analyzed for water by this scheme.



Ten cc. of 1.5 molal acetyl chloride in toluene is pipetted into a dry 250 cc. glass stoppered volumetric flask, using an automatic pipette. The flask is then placed in a beaker containing a slurry of finely chopped ice, and after standing for a minute or so, 2 cc. of pyridine is added from a pipette. The mixture is stoppered and shaken. A thin paste of acetyl pyridinium chloride in toluene is formed. The known weight or volume of sample to be analyzed for water content is added in such proportions

[10] Smith and Bryant, *J. Am. Chem. Soc.* **57, 841** (1935).

that an excess of 0.5 mole of acetyl chloride remains for each mole reacted. The mixture is shaken vigorously to ensure intimate contact between the reagent and the sample. After standing for at least two minutes at room temperature, in which time all of the water present should have reacted, the excess of reagent is decomposed by absolute ethanol added in two installments. The first 1 cc. of absolute ethanol is added from a pipette and followed by vigorous shaking to decompose the major portion of the reagent. Then after at least 5 minutes, 25 cc. more of absolute ethanol is added to complete the decomposition and produce a homogeneous solution suitable for titration. The solution is allowed to stand an additional ten minutes at room temperature before titration. The addition of absolute alcohol in two portions is necessitated by its small but nevertheless significant water content which would otherwise introduce errors of varying magnitude.

The mixture is then titrated with 0.5 *N* sodium hydroxide solution to a phenolphthalein end-point. At least one blank determination should be made along with each group of samples. The increase in acidity of the sample over the blank is a direct measure of the water present in the sample, one mole of water liberating an extra mole of acid.

Determination of Ash

Ash is the residue remaining after a foodstuff is ignited until it is carbon free, usually at a temperature not exceeding red heat.

Weigh into a tared platinum or porcelain or other suitable dish, a quantity of substance representing 2 g. of the dry material. Dry, burn at a low red heat and then ash in a muffle oven at a dull red heat until free from carbon. If a carbon free ash cannot be obtained, leach the ash with water, filter on ashless filter paper. Return the filter and residue to the dish and burn to a white or nearly white ash. Cool, add the filtrate, evaporate to dryness and heat at dull red heat until a white or nearly white ash is obtained. Do not ash materials high in phosphates or containing lead, arsenic or antimony in a platinum dish, for such ashing may make the platinum brittle. A small heat resistant glass beaker [11] may be used advantageously for ashing in preference to both porcelain or platinum, especially where the temperature of the ashing is low and where an ash that might fuse into the porcelain is likely to be obtained.

[11] Pyrex beakers made by Corning Glass Works are suitable.

It is advantageous, sometimes, to combine the direct moisture determination and ash determination by first drying the material at 100–105° C., weighing the loss in moisture and then performing the ash determination.

Water Soluble and Insoluble Ash—Add water to the ash obtained as directed above and heat almost to boiling. Filter through an ashless filter paper and wash with hot water until the combined filtrate and washings measure about 60 cc. Reserve the filtrate and washings for the determination of alkalinity of soluble ash. Return the filter paper and its contents to the original dish in which the ashing was performed, ignite carefully, cool, and weigh. From the respective weights found, the percentage of water soluble and insoluble ash may be calculated, for the total ash minus insoluble ash equals the soluble ash.

Alkalinity of Ash—Cool the filtrate and washings reserved as directed immediately above and titrate with 0.1 *N* hydrochloric acid, using methyl orange indicator solution, made by dissolving 1 g. of the dye in a liter of water. Alkalinity of ash is generally expressed as the number of cc. of normal acid per 100 g. of sample. This determination gives the alkalinity of the water soluble ash.

To estimate the alkalinity of the total ash or water insoluble ash, add an excess of 0.1 *N* hydrochloric acid to the dish containing the ash and heat to boiling cautiously on a hot plate. Cool, and titrate the excess hydrochloric acid with 0.1 *N* sodium hydroxide solution, using methyl orange as indicator. The alkalinity may be expressed in terms of the number of cc. of normal acid per 100 g. of sample or as the alkalinity number, which is defined as the number of cc. of normal acid required to neutralize 1 g. of ash.

Determination of Nitrogen

Another usual analysis is the determination of nitrogen, for from the nitrogen content, the protein content of materials may be calculated. Proteins are complex organic substances consisting of chains of amino acids. They are a major constituent of all living cells, both plant and animal. As was previously discussed in the general introduction, proteins are necessary components of animal foods. The nitrogen content of different proteins is nearly alike and is approximately 16 per cent,

hence multiplying the nitrogen estimated by the factor 6.25 yields the amount of protein. In certain cases as, for example, casein, a higher factor, namely, 6.38 is used for this conversion and more nearly represents the true proportion of nitrogen in those cases.

The estimation of nitrogen is generally done by a modified Kjeldahl digestion method. This digestion should be done only in a hood with a good draught. This method depends upon the decomposition of organic nitrogen compounds by boiling with sulfuric acid. The carbon and hydrogen of the organic material are oxidized to carbon dioxide and water. A part of the sulfuric acid is simultaneously reduced to sulfur dioxide which in turn reduces the nitrogenous material to ammonia. The ammonia combines with the sulfuric acid and remains as ammonium sulfate, a substance with a high boiling point. The ammonia is subsequently liberated by the addition of sodium hydroxide; is distilled into a known amount of standard acid and the excess acid is estimated by titration with standard alkali. In the method detailed, that is, the Kjeldahl-Gunning-Arnold Method, copper sulfate or mercury is added to act as a catalyst. Potassium sulfate or sodium sulfate is added in order to raise the temperature of the reaction mixture and thus hasten the digestion.

Gerritz and St. John[12] recommend the addition of 10 g. of anhydrous dipotassium phosphate or 12 g. of dipotassium phosphate trihydrate to be substituted for 10/16ths of the potassium or sodium sulfate to obtain more rapid digestion.

Kjeldahl-Gunning-Arnold Method Transfer a weighed portion of about 0.7–3.5 g. of the material according to its nitrogen content into a digestion flask, Fig. 10. This may be done by weighing by difference or by weighing the material directly on filter paper or ungummed cigarette paper and transferring the paper and its contents to the flask. If the material is moist, a convenient method is to support a weighed piece of filter paper on the balance pan by means of a watch-glass and rubber washer of a mason jar. Then weigh the material directly and rapidly on the filter paper and after recording the weight, transfer paper and contents to the flask. The rubber washer prevents the filter paper from touching the watch-glass and thus prevents any loss of moisture by wetting the watch-glass.

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[12] Gerritz and St. John, *Ind. Eng. Chem., Anal. Ed.* **7**, 380 (1935).

Add 15–18 g. of anhydrous potassium sulfate or anhydrous sodium sulfate or an equivalent amount of the crystallized hydrated salts, 1 g. of crystallized copper sulfate, $CuSO_4.5H_2O$, or approximately 0.7 g. of mercuric oxide, HgO, or its equivalent of metallic mercury and 25 cc. of sulfuric acid. Do not add mercuric oxide or mercury if it is possible to carry out the digestion easily without these materials, for then the subsequent addition of potassium or sodium sulfide solution may be avoided. Heat the mixture gently until frothing ceases, then boil briskly and continue the digestion for a time after the mixture is colorless or nearly so, or until the oxidation is complete. The digestion usually requires at least 2 hours and the flask should be rotated at intervals during the digestion. Cool, add about 200 cc. of water, and if mercuric oxide or metallic mercury has been used, add also 50 cc. of potassium sulfide solution, 40 g. K_2S per liter, or sodium sulfide solution, 40 g. Na_2S per liter, or sodium thiosulfate solution, 80 g. $Na_2S_2O_3.5H_2O$ per liter. Then make strongly alkaline by pouring 70–75 cc. concentrated sodium hydroxide solution, 454 g. $NaOH$ plus 1 liter of water, down the side of the flask so that it does not mix at once with the acid solution. Add a pinch of zinc dust to prevent bumping and reduce frothing. Connect the flask to the condenser by means of a Kjeldahl connecting bulb, taking care that the tip of the condenser extends below the surface of the standard acid in the receiver and that the contents of the flask are mixed completely by shaking the flask at first carefully and cautiously and then vigorously. Distill until all of the ammonia has passed over into a measured quantity of standard acid. The first 150 cc. of the distillate will generally contain all the ammonia.

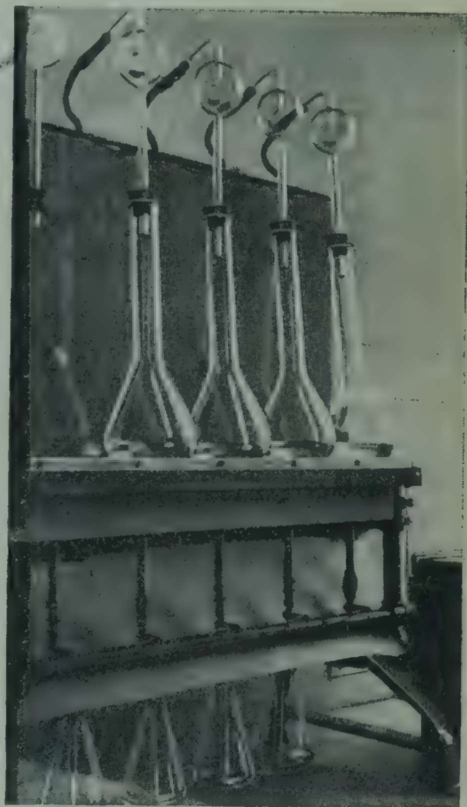

FIG. 10. Kjeldahl Nitrogen Apparatus

Titrate with standard alkali using methyl red indicator, 1 g. of the dye in a mixture of 50 cc. 95 per cent alcohol plus 50 cc. of water.

The Winkler [13] modification of the Kjeldahl method is very useful. In this method the ammonia is distilled as usual but is fixed in 50 cc. of a saturated solution of pure recrystallized boric acid with the formation of ammonium borate. The ammonia may then be titrated directly with standard acid, because the boric acid is too weak an acid to affect the hydrogen ion concentration to an appreciable extent during the titration. The advantages of this method are that it needs only one standard solution, namely, acid; it saves time and the boric acid need be measured only approximately. Care must be taken, however, that the receiver of the distillate be kept cool during the distillation for ammonium borate is somewhat volatile.

Farinacci Modification of the Koch and McMeekin Method—As an example of many types of micro nitrogen determinations the Farinacci [14] modification of the Koch and McMeekin [15] method is detailed. It is a direct nesslerization method in which the organic material is destroyed by digestion with sulfuric acid and 30 per cent hydrogen peroxide and a portion of the resulting solution is nesslerized and compared with prepared standards in a colorimeter.

Transfer a weighed portion of material according to the nitrogen content, from 0.25 g. of a meat product to 5 cc. of a low nitrogen content material such as orange juice, to a 250 mm. x 25 mm. lipped Pyrex test tube. Cigarette paper is most conveniently used for this type of transfer, especially where practically dry materials are being used. Care must be taken to cut away the gummed portion of the cigarette paper.

Add 2 cc. of sulfuric acid (1 : 1) and 6 drops of copper sulfate solution, 1 g. $CuSO_4.5H_2O$ in 100 cc. water, and if a dry material is being used, 2 cc. of water. Place the tube in a clamp at an angle, add two glass beads, boil off the water with the aid of a micro burner and continue heating until fumes of sulfur trioxide fill the bottom of the tube. Adjust the tube to a vertical position and add 2-3 drops of 30 per cent hydrogen peroxide solution to the hot mixture. Heat again with the micro burner until the fumes are produced, remove the flame for 30 seconds and again add 2-3 drops of hydrogen peroxide. Continue the alternate heating

[13] Winkler, *Z. angew. Chem.* 26, 231 (1913).

[14] Farinacci, personal communication (1935).

[15] Koch and McMeekin, *J. Am. Chem. Soc.* 46, 2066 (1924).

and addition of hydrogen peroxide until the digestion is complete. During the alternate heating and addition of the peroxide, the solution will become clear almost immediately after the addition of the peroxide and the further heating will cause the mixture to darken again. Digestion is complete when no further darkening occurs on continued heating, and the solution is perfectly clear. The process is usually complete in about 20 minutes. As much as 30 drops of the peroxide are needed at times. The tube is cooled. Transfer the contents to a 100 cc. volumetric flask. Make to volume. Transfer from one to 5 cc., according to the nitrogen content, to a 50 cc. volumetric flask, dilute to 35 cc., add 6 cc. of Folin's Nessler reagent, prepared as directed immediately below, and make to volume. Read in a colorimeter against a standard prepared by diluting 10 cc. of ammonium sulfate solution containing 471.6 mg. per liter to 100 cc. Pipette 20 cc. of this solution into a 50 cc. volumetric flask, dilute to 35 cc., add the same quantity of Folin's Nessler reagent as above and make to volume. Each cc. of the standard solution now contains 0.004 mg. of nitrogen. If the nitrogen content of the unknown is much higher than the standard, less than 4 cc. of the unknown is nesslerized and conversely, if the nitrogen content is much lower than the standard, more than 4 cc. is nesslerized.

Care must be taken not to heat the tube too strongly when sulfur trioxide fumes are produced for some ammonium sulfate may be lost mechanically by bumping or by other means.

Preparation of Folin's Nessler Reagent

Nessler's solution is an alkaline solution of the double iodide of mercury and potassium[$HgI_2.2KI$]. Transfer to a 200 cc. flask 30 g. of potassium iodide and 22.5 g. of iodine; add 20 cc. of water and after solution is complete, an excess of metallic mercury, that is, approximately 30 g. Shake the flask continuously and vigorously until the dissolved iodine has nearly all disappeared which takes about 7 to 15 minutes. The solution becomes hot. When the red iodine solution has begun to become visibly pale, though still red, cool in running water and continue shaking until the reddish color of the iodine has been replaced by the greenish color of the double iodide. The whole operation generally takes 15 minutes. Test a portion of the solution with starch solution. Unless the starch test is positive, the solution may contain mercurous compounds. Decant the solution, washing the mercury and flask with water. Dilute the solution and washings to 200 cc. and mix well. If the cooling was

begun in time, the resulting reagent is clear enough for immediate dilution with 10 per cent alkali and water and the finished solution can be used at once for nesslerization. From this stock solution of potassium mercuric iodide, prepare the final Nessler's solution as follows: To 975 cc. of an accurately prepared 10 per cent sodium hydroxide solution, add the 200 cc. of the double iodide solution. Mix thoroughly and allow to clear by standing.

The 10 per cent sodium hydroxide solution should be made from a 1 : 1 solution of sodium hydroxide and water which has been allowed to stand until the carbonate has settled, the clear solution being decanted and used. This solution should be standardized to an accuracy of at least 5 per cent by titration and subsequent adjustment by the addition of more water or alkali as the case may be. The alkalinity of Nessler's reagent is important and should be checked against *N* hydrochloric acid. Twenty cc. of *N* hydrochloric acid should require 11 to 11.5 cc. of Nessler's solution.

Determination of Nitrogen Including Nitrates—Any nitrogen present in a sample in the form of nitrate would be lost as nitric acid by volatilization during the Kjeldahl digestion. When it is desired to include this form of nitrogen, the Kjeldahl-Gunning-Arnold method must be modified. This may be done by adding some substance such as salicylic acid or phenol, which is readily nitrated and holds the nitric acid formed as the nitro-derivative. The nitro group is then subsequently reduced to an amino group and finally to ammonia.

To the weighed sample transferred to a digestion flask as directed above, add 30 cc. of sulfuric acid containing 1 g. of salicylic acid, shake until thoroughly mixed, and allow to stand for at least 30 minutes with frequent shaking or until complete solution results. Add 5 g. of crystallized sodium thiosulfate and digest as directed below. As an alternate procedure, add to the substance 30 cc. of sulfuric acid containing 2 g. of salicylic acid, allow to stand at least 30 minutes with frequent shaking or until complete solution results. Add gradually 2 g. of zinc dust, shaking the contents of the flask at the same time and digest as follows: Heat over a low flame until all danger from frothing has passed. Then increase the heat until the acid boils briskly and continue the boiling for 5–10 minutes or until white fumes no longer escape from the flask. Cool, add 10 g. of potassium sulfate or anhydrous sodium sulfate and proceed as directed in the Kjeldahl-Gunning-Arnold Method.

Extractions and Separations

One operation performed very often in food analysis is that of extraction and subsequent separation. Thus in nearly every chapter in this book a procedure is detailed in which extractions either by immiscible solvents or by continuous methods are made.

The fundamental law governing the process of extraction, more particularly known as the law of partition, is a special case of Henry's law which states: The concentrations of any single molecular species in two phases at equilibrium bear a constant ratio to each other, the temperature remaining constant. As applied to the special case of its use in food analysis, the law is more simply stated: A solute will distribute itself, at constant temperature, between immiscible solvents in a definite manner which depends on the solubility of the substance in each of the solvents separately. This is conditioned by the fact that the distribution of the solute is also governed by its molecular weight and species in each solvent. A distribution in which the solute has the same molecular weight and species in both solvents is stated mathematically:

$$\frac{c_1}{c_2} = \text{coefficient of partition} = \text{constant} \qquad \text{in which,}$$

c_1 = concentration in moles per liter in the first solvent.

c_2 = concentration in moles per liter in the second solvent.

In many cases in food analysis, the solute is not in the same molecular state in both solvents. Thus for example, in the distribution of benzoic acid between water and benzene, the benzoic acid is in the form of dimeric molecules in the benzene. Then for such a system the concentration of the single molecules in the second solvent $nA \rightleftarrows A_n$ would be according to the law of mass action proportional to the nth root of the total concentration, and therefore if c_1 is the concentration of the solute in the 1st solvent and c_2 is the concentration in the 2nd solvent, the mathematical statement becomes

$$\frac{c_1}{\sqrt{c_2}} = \text{coefficient of partition} = \text{a constant.}$$

It can be seen then, that the solute will merely distribute itself between the two solvents in proportion to its solubility as explained above and that therefore it will, in general, be necessary to make repeated extractions with the second solvent before the concentration of the solute in the first solvent is reduced to a negligible quantity.

The most common form of apparatus used for extractions and separations is the separatory funnel, which is well adapted for multiple extractions by one solvent which has a higher specific gravity than another solvent. Thus for example, in the extraction of lead from an aqueous ammoniacal cyanide solution by a solution of dithizone in chloroform, repeated extractions may be made by chloroform portions without removing the aqueous layer from the separatory funnel.

Jacobs-Singer Separatory Flask—The separatory funnel is not well adapted for multiple extractions in which the supernatant layer, that is the lighter specific gravity liquid is the extracting medium for the higher specific gravity layer must first be drawn off through the stop-

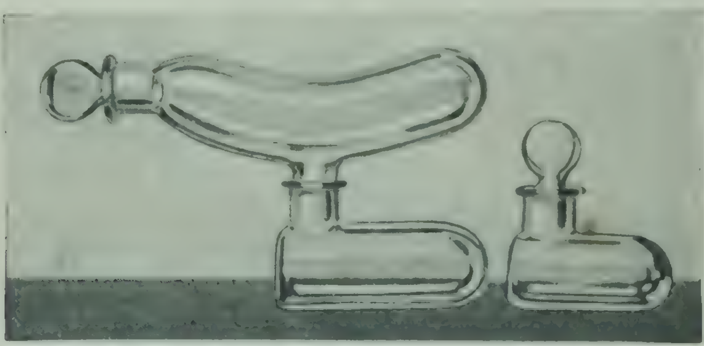

FIG. 11. Jacobs-Singer Separatory Flask

cock and then the lighter layer is generally poured through the upper orifice in order to reduce contamination.

There are several types of apparatus designed to achieve this purpose. One of the most useful is the Jacobs-Singer separatory flask. This apparatus, Fig. 11, consists of two sections either separable or integral substantially horizontal to one another. If separable, the lower section is an independent flask and is so designed as to permit direct weighing without the use of any auxiliary attachments and to permit its use as a chemical vessel in ordinary operations. The lower section, when separable, is fitted with an offset orifice or opening ground with standard taper to fit not only the upper section but also because of the interchangeable joint, the stopper or any other piece of apparatus, such as a condenser, having the same taper. The lower section is also fitted with a flange or shoulder to aid in the handling of the apparatus. Furthermore, it has a flat bottom so that this section of the flask can, as the illustration shows, support the upper section.

The upper section of the flask is a curved tube having two orifices. One, at the lower portion of the middle, is externally ground to fit into the lower section as if it were a stopper. The upper section of the flask is so designed that there is a downward slope from both ends to the middle orifice. One of the ends has a pouring orifice which is internally ground at the same standard taper.

The use of standard taper permits the interchange of upper and, more often, lower sections of divers capacities as illustrated in Fig. 11. Thus, as shown in the illustration, the glass stoppers, the upper section and the two lower sections are all interchangeable. The more convenient capacities for general analytical work consist of an upper section of 100 to 120 cc. and lower sections of 25 to 30 cc. and 55 to 60 cc. capacities, respectively.

The general method of use of this separatory flask is the following. The material to be extracted is either weighed directly into the lower section, or a weighed portion is transferred to this section by means of a weight pipette or by weighing by difference, or an aliquot is placed in the flask by a suitable means such as a pipette, burette or cylinder, depending on the accuracy desired. The sample in the flask is then subjected to whatever processing is required in the method, for example see the Roese-Gottlieb method for fat in cheese, or the ethyl ether method for unsaponifiable matter in oils or the separation of colors by the immiscible solvent method, etc. Then the upper section is placed in position and the remaining agents added or else the appropriate solvent is added, until the lower level of the connecting joint is reached. The amount of additional solvent to add depends on whether after shaking with the extracting solvent, the volume of the lower liquid, that is the heavier specific gravity solvent is increased or decreased due to the respective solubilities of the one solvent in the other. Then the requisite amount of extracting solvent is added.

The stopper is inserted in the outlet or orifice of the upper section. Now holding the upper section in the palm of the hand and holding the lower section firmly against the upper section by means of the fourth and fifth fingers, while at the same time holding the stopper firmly with the thumb, the liquids are separated so that some of each solvent is in each section of the flask, by a few deft movements and then the contents of the flask are thoroughly shaken. If a volatile extractive solvent is being used, after shaking, hold the flask with the outlet upward and relieve the pressure by removing the stopper. Restopper, set the apparatus on its base and allow the liquids to separate.

The higher specific gravity liquids will of necessity flow to the bottom flask due to the taper of the upper section. After the separation is complete, the supernatant liquid may be poured out by simply removing the stopper and slightly tilting the flask. The extraction may now be repeated in the same manner the required number of times. At the end of the extraction, any of the extracting solvent left in the lower section of the flask or in the lower part of the connecting joint, may be removed by adding more of the lower solvent, cautiously to avoid mixing and over-shooting

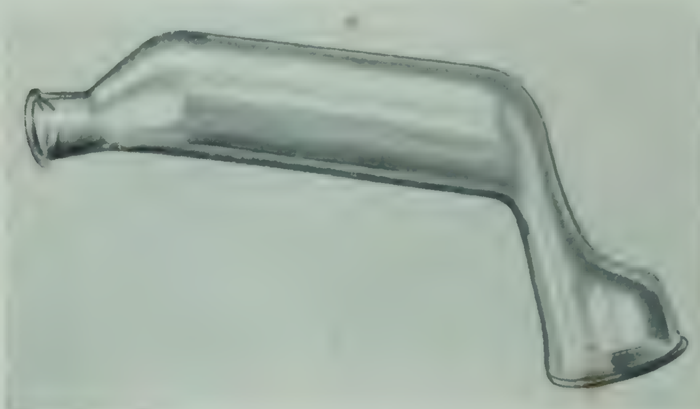

FIG. 12. Mojonnier Extraction Tube

the mark, from a wash bottle, thus raising the level of the extracting liquid high enough in the connecting joint to be poured off.

If very stubborn emulsions form, pass all of the liquid into the upper section after stoppering the orifice. Invert this section, remove the lower section and after stoppering the middle orifice by means of a rubber nipple, centrifuge by placing the upper section in a suitable centrifuge cup.

If series of extractions are being made, a box designed to hold a number of these separatory flasks so that they may all be shaken at once is very useful.

Mojonnier Extraction Tube[16]—Another device used for multiple extractions with supernatant layers is the Mojonnier extraction tube, Fig. 12. This device is of limited use being mainly devised for use as

[16] Mojonnier and Troy, "Technical Control of Dairy Products," Mojonnier Bros. (1925).

the extraction apparatus needed in the Roese-Gottlieb method for the estimation of fat in milk products. This extraction apparatus consists of a tube having two chambers, a mixing chamber and a settling chamber at an angle to one another and connected by a constriction in the tube. The material to be analyzed is weighed into the flask which must be suspended from hooks in the balance or else a definite quantity of sample or rather quantity or volume of it is transferred to the tube from some auxiliary apparatus. The various reagents required by the method are added, the tube is stoppered with a cork stopper and then shaken. The extracting solvent is then added and again the tube is stoppered and shaken and the solvents are permitted to separate. When separation is complete, the supernatant layer can be poured off through the mouth of the tube without disturbing the lower layer. Exact details are to be found in other sections of the book, see particularly the Roese-Gottlieb method for milk.

The flask or rather tube must be equipped with some box holder for it cannot stand independently. The same box can be used to hold an entire series of tubes and thus aid in routine analyses.

Soxhlet Continuous Extraction Apparatus—In contradistinction to the extraction process in which multiple portions of an extracting immiscible solvent are used, there is the continuous extraction process. In this procedure, the apparatus is so designed that a fresh portion of solvent comes in contact with the material to be extracted over a relatively long period of time. There are many forms of this type of apparatus. One of the most commonly used is the Soxhlet type of continuous extractor, Fig. 13.

The apparatus consists of a tared flask, containing a volatile solvent and resting on some type of heating device, generally and preferably a multi-regulated electric hot plate. The tared flask is connected preferably by means of interchangeably ground joints with a tube having a siphon arrangement and side arm. The extraction tube is connected, again preferably by interchangeably ground joints with a condenser. The apparatus is used in the following manner. The dry material is weighed into or a weighed amount of dry material is transferred to a thimble which may be of alundum, cotton or other porous material. The thimble is placed in the extracting tube and this tube is then connected with the weighed flask and also the condenser.

The heat vaporizes the volatile solvent which passes up the side arm

and is condensed in the condenser. The condensed solvent falls drop by drop into the thimble. When sufficient solvent has been thus transferred to the extracting tube to fill the siphon arm, it siphons back over into the weighed flask. This process is continued until the extraction is

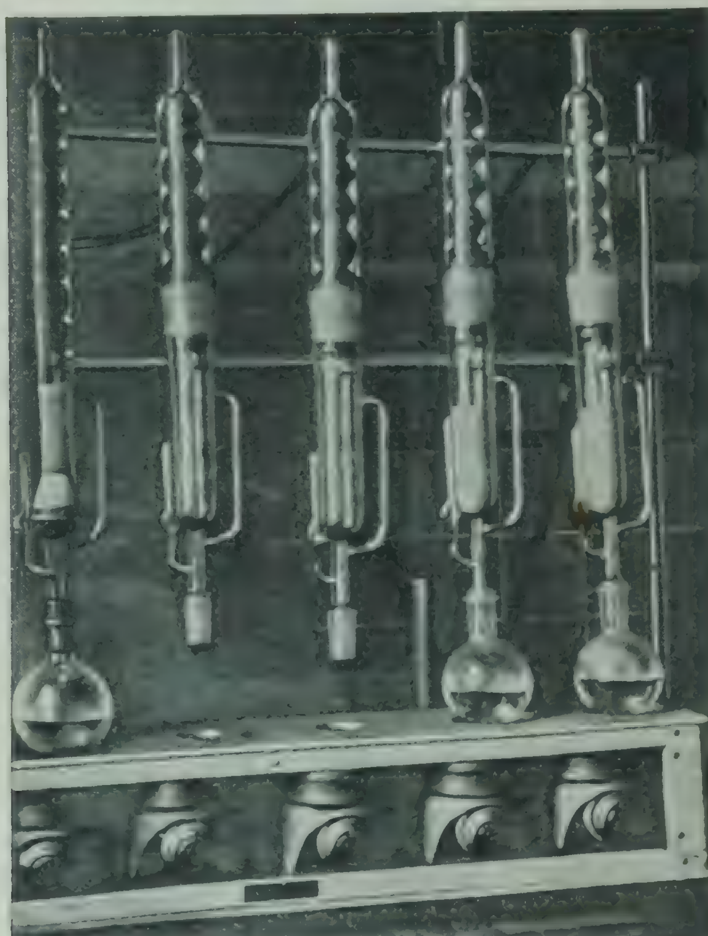

Fig. 13. Soxhlet Extraction Apparatus

complete, varying at times from 8 to 24 hours. Then the tared flask is removed, the volatile solvent is evaporated and the residue is the extracted material.

For other types of continuous extractors see Blasdale[17] or some similar text.

[17] Blasdale, "Fundamentals of Quantitative Analysis," Van Nostrand (1928).

Organoleptic Analysis

It is often the function of the food analyst to analyze a food product organoleptically, that is, by the use of his senses. The taste, odor, and appearance of a food product are very often indicative of the quality of that product. Naturally, discretion must be observed in the interpretation. Thus although a fruit would be considered bad food if it were slimy or moldy or had a putrid odor, certain types of cheese, as is well known, may have all of these characteristics and still be fit food.

The general intent behind organoleptic analysis is to obtain some estimate of the taste, odor, and appearance of the product; to note if it be wormy, moldy, if it contain worm excreta or worm tracks; whether it be rancid or of foul odor or whether it be contaminated with any extraneous matter such as insects, mouse excreta, mouse hair, etc. Nuts, dates, figs, cocoa beans, coffee beans, legumes, olives, fruits and many other food products are conveniently examined by this method.

The objectionable features of food materials may be classified and described as follows:

1) Wormy. Food products may be considered wormy if they show evidence of worms, their excreta, their infestation or their tracks.

2) Shriveled or empty. Foods, such as nuts or fruits, may be considered shriveled or empty if more than one-half of the kernel or contents are entirely withered or if the contents are shrunken to more than one-half of the cavity or interior or if more than one-half of the interior is dry.

3) Moldy. A foodstuff may be considered moldy if it shows definite evidence of hairy masses of mycelium.

4) Decomposed or rancid. Food products may be considered decomposed or rancid if they show definite evidence of decomposition and spoilage. This is characterized by the presence of foul, putrid or rank odor, marked discoloration, slimy feel and appearance, decayed texture, soft spots, rot, rancid taste and similar characteristics.

5) Dirty or filthy. Foodstuffs may be considered dirty or filthy if dirt, filth, or extraneous matter, such as insects, parts of insects, hair and excreta of animals and the like are present.

6) Miscellaneous objectionable features. Any other objectionable feature in a food material such as bitter taste in almonds, salty or sour taste in milk, cheesy, yeasty or fruity taste in butter or any other

unnatural concomitant taste, odor or appearance of a food may be placed in this classification.

Systematically, this type of analysis may be made by selecting a given number, say 100, of pieces of a properly sampled material, breaking, cutting open, tasting, smelling, observing with the aid of a hand lens, and otherwise examining the pieces and then noting the number in each group listed above. The total number in each classification type may then be expressed in terms of percentage. If this number examined shows the foodstuff to be substandard, a greater number of pieces should be examined to substantiate the first findings.

Canned goods are frequently examined organoleptically. The analyst should note the external appearance of the containers to detect the presence of "swells", "springers" or "leakers".

Swells are canned goods in which decomposition has taken place so that the formation of gas causes the ends to bulge and be convex instead of the normal condition, namely, slightly concave.

Springers are canned goods which may appear normal in ordinary weather but bulge in hot weather. There is little doubt this condition results from improper canning.

Leakers are canned goods which have openings or orifices from which the contents of the can are able to escape.

After opening the can, the odor, appearance, color, flavor and size of the food material should be noted. The liquid or brine should also be examined, to see if it be clear or turbid. Any variation from the normal, which the analyst knows from experience, will generally be evident. If it is desired, the gases in the can may be collected, before opening the can, with the aid of a Doremus gas collector.[18] Macroscopic and microscopic examination can then be made according to the classification listed above.

The container, itself, should be examined for evidence of corrosion or blackening. The distance of the height of the food material and liquor from the top seam should be noted to detect slack filling.

The examinations described in the preceding part of this section help to determine if a food be adulterated or deleterious. However, organoleptic examination may be made to ascertain if a food be standard or substandard, though still good food.

The Food and Drugs Act of 1906 was amended in 1930 to grant control to the U. S. Department of Agriculture, over the standards and

[18] Leach-Winton, "Food Inspection and Analysis," Wiley (1920).

fill of canned goods except of milk, and meat and meat products. Such standards are promulgated in Service and Regulatory Announcements, Food and Drug No. 4, 4th rev. (1937) of the Food and Drug Administration, U. S. Department of Agriculture. These regulations govern canned food standards and not adulterated or deleterious foods.

In order to ascertain if the canned food is standard or substandard, though still good food, according to these promulgations and definitions, the following factors are examined and evaluated.

1) Color—The food material is "normally colored" if it has a naturally developed general effect of color. If the color is designated by a system, it must conform to that system. (See Munsell Color System, Chapter II.)

2) Size—(a) The food material is "normal sized" if the units are of the designated size or over.

(b) The food material is "uniform sized" if the weight of the piece of largest size in the can be not more than twice the weight of the smallest piece in the can.

3) Tender—The food material is "tender" when it complies with the definitions referred to above. An apparatus for determining the tenderness of canned fruits and vegetables by crushing and penetration tests is described by Bonney and Lepper.[19]

4) Peeled—The food material is "peeled" if there is present per pound of net contents not more than a prescribed area of peel. In the case of peaches and pears, 1 square inch and in the case of tomatoes, 3 square inches are the prescribed amounts.

5) Unblemished—The food material is "unblemished" if 80 per cent or more of the pieces in the container are free from unsightly scabs, bruises, frostbites, sunburn, hail injury, cracks, checks, raggedness (frayed condition of the edges), unnatural colorations or other unsightly blemishes.

The standards deal mainly with fruits and vegetables, consequently the aforementioned descriptions deal with canned fruits and vegetables.

REFERENCES

Findlay, "Practical Methods of Physical Chemistry," Longmans, 5th ed. (1933).
Mahin, "Quantitative Analysis," McGraw-Hill (1924).

[19] Bonney and Lepper, U. S. Dept. Agr., Circ. 164 (1931).

Fryer and Weston, "Oils, Fats, and Waxes," Cambridge (1920).
Methods of A. O. A. C., 4th ed. (1935).
Woodman, "Food Analysis," McGraw-Hill, 3rd ed. (1931).
Leach-Winton, "Food Inspection and Analysis," 4th ed., Wiley (1920).
Moore, "History of Chemistry," McGraw-Hill (1918).
Sherman, "Chemistry of Food and Nutrition," 5th ed., Macmillan (1937).
Brooks, "Critical Studies in the Legal Chemistry of Foods," Chemical Catalog (1927).
Blasdale, "Fundamentals of Quantitative Analysis," Van Nostrand (1928).

CHAPTER II

PHYSICAL CHEMICAL METHODS

Physical Chemical methods are of growing importance in food analysis. They lend themselves to rapid, simple means of determining with a great degree of accuracy many factors we are anxious to estimate. For example, it takes hours or days sometimes to determine the per cent total solids or conversely the per cent moisture in sugar solutions whereas with the use of a refractometer it is a matter of minutes. It is beyond the scope of this text to describe in great detail any and all of these instruments. However, the principles upon which some of them are based will be outlined and the use that they find in food chemistry will be indicated.

Refractometry

The refractive index is a quantity which is a constant for a pure substance under standard conditions of temperature and pressure. It is the ratio of the sine of the angle of incidence of a ray of light on the surface separating two media to the sine of its angle of refraction. The ray passing from a dense to a denser medium is bent towards the normal. Expressed mathematically,

$$\frac{\sin i}{\sin r} = n = \text{index of refraction}$$

Abbé Refractometer—With the Abbé refractometer, Fig. 14, the refractive index can be read directly, only a few drops of the liquid are needed and either white or ~~monochromatic light~~ can be used. This refractometer consists, mainly, of a fixed telescope and two matched right angle prisms. The liquid is placed in contact with the prisms. A ray of light passing through the prism and liquid and entering the prism ABC at grazing incidence, Fig. 15, will emerge from AC at less than a right angle for any value of n other than

$$n = \sin A.$$

Hence, the prism must be rotated through an angle in order that the rays may be parallel to the telescope. This angle of emergence determines the refractive index. Rays striking the surface at an angle greater than the angle of emergence are totally reflected. By adjusting the light and dark portions of the field so that the line of demarcation is sharp and coincides with the cross hairs, the refractive index can be read directly on the scale. The prism box has a jacket so that the temperature

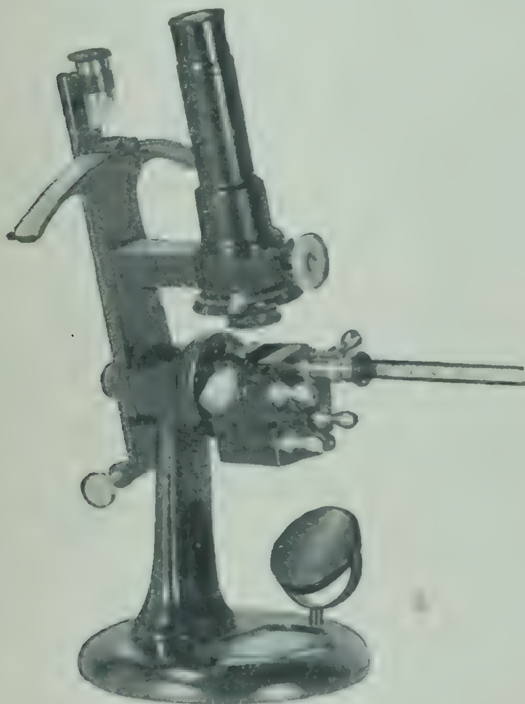

FIG. 14. Abbé Refractometer (Courtesy of Bausch & Lomb)

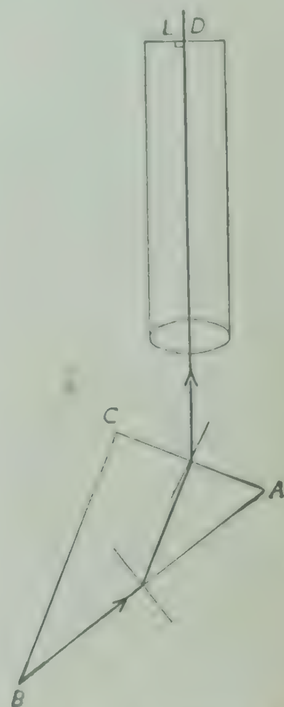

FIG. 15. Diagram of Refractometer Prism

can be controlled and the telescope has a set of Amici prisms, Fig. 21, for compensating light aberration.

Since the index of refraction of a pure substance is constant at constant temperature and pressure, it can be used as a means of identification. It is used to determine the purity of oils, fats and waxes, which, though not pure substances in the strict sense of the word, have indexes which vary over a slight range. It is used to determine the amount of sugar in sugar solutions and, in general, for determining total solids where sugar is a main constituent, as in fruit juices, tomato products,

honey, syrups and soda water. Methods have been developed for the determination of the percentage fat in cacao products, the percentage sugar in chocolate products and the estimation of total solids in eggs, all of which will be referred to in their appropriate section of the book.

Immersion Refractometer—The theoretical considerations of the immersion or dipping refractometer are the same as those of the Abbé refractometer. Hence in this instrument, also, determination of refractive index depends on the observation of the line of total reflection. It is an instrument designed to cover a small index range with a higher degree of accuracy than is possible with the Abbé type. It consists of three essential units, a telescope, a compensator and an immersion prism. Originally the immersion instrument was designed to read indices only from 1.32 to 1.36. However, by using interchangeable prisms the range of the instrument may be extended up to 1.54. This type of instrument gives greater accuracy than any other type refractometer except interference refractometers. It requires sufficient liquid to cover the prism after immersion.

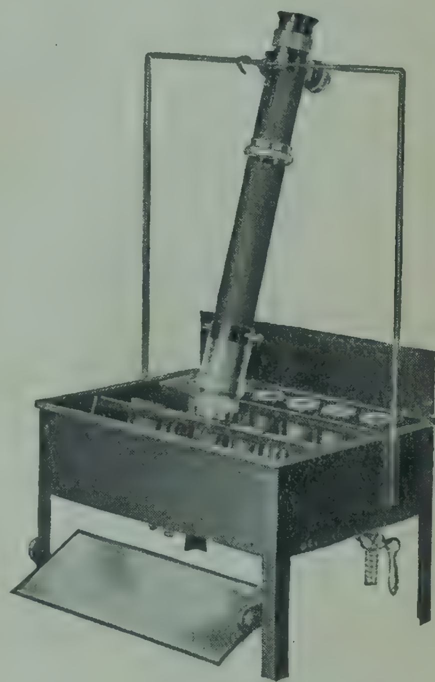

FIG. 16. Immersion Refractometer
(Courtesy of Bausch & Lomb)

The prism, Fig. 16, dips into the liquid to be measured in a cup which rests in a bath, so that the temperature may be carefully controlled. The light reflected from the mirror and passing through the prism is compensated by the internal Amici prism and illuminates the upper portion of the field of view of the telescope. The lower portion of the field of view is dark because it is not illuminated. This portion of the light is totally reflected. The instrument is focused until the line of demarcation is sharp. If the line does not coincide with a scale reading, the micrometer screw is set at 0, the next lower scale division is read and then the micrometer screw is turned until the line coincides with a scale division. The vernier reading is added to the scale reading and the

refractive index is obtained from Table 1 or Table 4 appendix. This instrument is used to determine the refractive index of milk serum, the per cent methyl alcohol present in ethyl alcohol and has many other uses.

TABLE 1. INDICES OF REFRACTION CORRESPONDING TO SCALE READINGS OF IMMERSION REFRACTOMETER

Scale Reading	n_D	Scale Reading	n_D	Scale Reading	n_D
0	1.32736	35	1.34085	70	1.35388
1	1.32775	36	1.34124	71	1.35425
2	1.32814	37	1.34162	72	1.35461
3	1.32854	38	1.34199	73	1.35497
4	1.32893	39	1.34237	74	1.35533
5	1.32932	40	1.34275	75	1.35569
6	1.32971	41	1.34313	76	1.35606
7	1.33010	42	1.34350	77	1.35642
8	1.33049	43	1.34388	78	1.35678
9	1.33087	44	1.34426	79	1.35714
10	1.33126	45	1.34463	80	1.35750
11	1.33165	46	1.34500	81	1.35786
12	1.33204	47	1.34537	82	1.35822
13	1.33242	48	1.34575	83	1.35858
14	1.33281	49	1.34612	84	1.35894
15	1.33320	50	1.34650	85	1.35930
16	1.33358	51	1.34687	86	1.35966
17	1.33397	52	1.34724	87	1.36002
18	1.33435	53	1.34761	88	1.36038
19	1.33474	54	1.34798	89	1.36074
20	1.33513	55	1.34836	90	1.36109
21	1.33551	56	1.34873	91	1.36145
22	1.33590	57	1.34910	92	1.36181
23	1.33628	58	1.34947	93	1.36217
24	1.33667	59	1.34984	94	1.36252
25	1.33705	60	1.35021	95	1.36287
26	1.33743	61	1.35058	96	1.36323
27	1.33781	62	1.35095	97	1.36359
28	1.33820	63	1.35132	98	1.36394
29	1.33858	64	1.35169	99	1.36429
30	1.33896	65	1.35205	100	1.36464
31	1.33934	66	1.35242		
32	1.33972	67	1.35279		
33	1.34010	68	1.35316		
34	1.34048	69	1.35352		

Colorimetry

Colorimetric analysis has advanced so much that it is an entire science in its own right. The fundamental law of colorimetry is Beer's law, which states that the absorption of light by solutions depends on the thickness of the layer traversed and on the molecular concentration in that layer. Hence, the transmitted light varies inversely as the concentration. This implies that the smaller the concentration, the greater the depth traversed for an equal absorption. Beer's law does not hold in all cases.

Colorimetry does not, of course, need a colorimeter. Matched tubes such as Nessler or Myer tubes are often used. A series of known standards are made and placed in the matched tubes and the color of the unknown, in another tube, is compared with that of the standards. The closest match gives the concentration of the unknown.

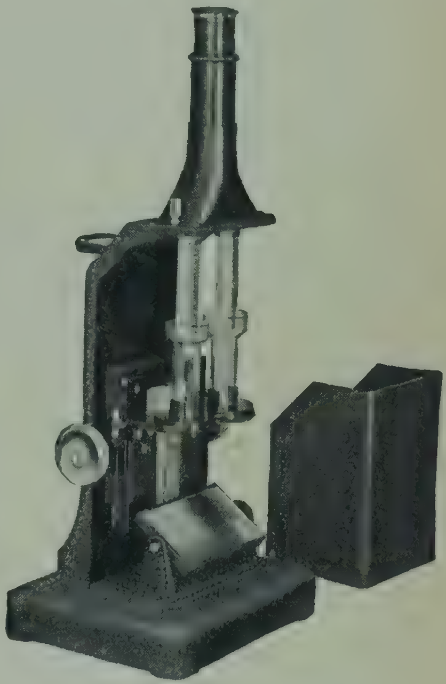

Fig. 17. Duboscq Colorimeter (Courtesy of Bausch & Lomb)

Colorimeter—There are many types of colorimeter used. One of the oldest and most frequently used is the Duboscq, Fig. 17. The instrument consists of 2 movable cups set on stages attached to rack and pinion, two plungers, a set of prisms, an eye piece and 2 millimeter scales with verniers. Diffuse light is reflected by a mirror through the cups and the plungers and is observed in the eye piece. One cup is partially filled with the unknown solution. The other cup is partially filled with the standard solution. The cups are raised so that the plungers dip into the liquid, care being taken that no air bubbles adhere to the plungers. Setting the standard at a definite position on the scale, usually at 15 or 20 mm., the unknown is moved up or down until the two halves in the eye piece appear equally colored. It is good practice to approach the point of equality a number of times from each direction and then average the readings. The light transmitted is now equal. Since the

transmitted light varies inversely as the depth and the concentration, we have mathematically for one side of the field:

$$\text{light transmitted for known} = \frac{1}{C_1R_1}$$

and for the other side of the field

$$\text{light transmitted for unknown} = \frac{1}{C_2R_2}$$

and since the light transmitted when the fields are equally colored is equal

$$\frac{1}{C_1R_1} = \frac{1}{C_2R_2}$$

Hence,

$$C_2R_2 = C_1R_1$$

and

$$C_2 = \frac{C_1R_1}{R_2}$$

where

C_1 = concentration of known
C_2 = concentration of unknown
R_1 = scale reading of known
R_2 = scale reading of unknown.

There are other types of colorimeters based on very similar principles.

Nephelometer[1] —A nephelometer is an optical device for determining the amount of suspended matter in a solution by comparison with standard suspensions. The method is based on the measurement of the brightness of the light reflected by the cloud, that is by the particles in suspension. The intensity of the light reflected is a function of the quantity of suspended particles when other conditions are constant.

[1] Kober, *Ind. Eng. Chem.* 10, 556 (1918).

Some colorimeters can be changed easily into nephelometers by changing cups and the source of light so that the light shines at the cups instead of through the cups. Hence, the amount of reflected light from the suspended particles in the liquids instead of the transmitted light may be measured by exposing varying amounts of the cup to the light. In a similar way then to colorimetry, nephelometry implies that the smaller the concentration, the greater the depth traversed for an equal reflection of light.

Photoelectric Colorimeter[2,3]—A photoelectric cell consists of a photo-responsive cathode surface of an alkali or alkaline earth metal or compound enclosed in a glass envelope which is either evacuated or filled with an inert gas under low pressure. The cell contains one or more anodes according to the design. Light falling on the active surface of this cell through its window will cause electrons to be hurled out of the active surface with a velocity depending on the frequency of the incident light and in number proportional to the intensity of illumination. The conditions are given by the well-known Einstein expression:

$$\frac{Ee}{300} = \frac{1}{2} mv^2 = h\nu - h\nu_0$$

where

E = potential in volts required to hold electron, e, at surface of emitter
m = mass of electron
v = velocity of emerging electron
h = Planck constant
ν = frequency of incident illumination
ν_0 = threshold frequency or lowest frequency at which electrons will be ejected.

If now an accelerating potential is applied between the active surface and the anode through a sensitive galvanometer, the electrons emitted from the active surface will travel across the interior of the cell to the anode and manifest themselves as a feeble current by causing a deflection of the galvanometer. Under proper conditions the reading on the galvanometer will be proportional to the intensity of the illumination reaching the active surface.

[2] Partridge, *Ind. Eng. Chem., Anal. Ed.* 2, 207 (1930).
[3] Partridge and Muller, *Ind. Eng. Chem., Anal. Ed.* 3, 169 (1931).

The photoelectric colorimeter, Fig. 18, consists essentially of an optical system, an absorption cell and an electrical measuring system. The optical system consists of a source of light in a well ventilated housing, a precision iris diaphragm, and a light filter with suitable transmission characteristics. The electrical measuring system consists of a photoelectric cell and a sensitive low resistance microammeter or a thermionic amplifier to amplify the photoelectric current derived as described above which may then be read on a milliammeter. The use of proper filters is important or preferentially monochromatic light should be used.

Fundamentally, this instrument measures the difference in light fall-

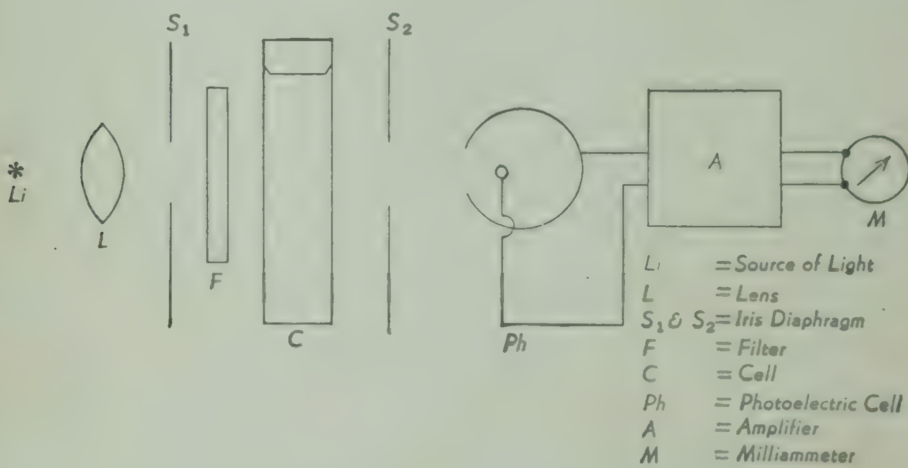

FIG. 18. Photoelectric Colorimeter

ing on a photoelectric cell due to different absorptive powers of liquids. Curves are plotted for known values of each particular absorptive substance, as for example, hydrogen ion indicators on semi-logarithmic graph paper. It can be seen from Beer's law:

$$T = t^{cl}$$

where

T = the transmission factor of concentration c in a thickness l

and

t = the transmission factor for a solution of unit concentration and unit thickness

and more readily from a more useful form of Beer's law

$$c = -\frac{1}{k}\log_{10}[I/I_0]$$

where

c = concentration of the substance in solution
I_0 = intensity of the incident light
I = the intensity of the light transmitted through a 1 cm. thickness of solution

that a logarithmic relationship exists between the concentration of the absorbing medium and the intensity as measured by the electrical measuring device. These curves act as calibration curves for each particular substance and are especially useful where Beer's law does not hold.

The following terms are often used in connection with absorption phenomena. The symbols and the defining equations are given. The defining equations are expressions in the typical form for the transmitted light according to the Beer law.

Extinction Coefficient = K	$I = I_0 10^{-lK}$
Molecular Extinction Coefficient = ε	$I = I_0 10^{-\varepsilon cl}$
Absorption Coefficient = μ	$I = I_0 e^{-\mu l}$

The photoelectric colorimeter is finding growing use in replacing and supplementing the visual colorimeter, for it is time saving and diminishes the personal factor in colorimeter readings. It is widely used in biochemical determinations and some instruments are equipped with scales reading percentage directly, for a particular substance, provided a fixed method is followed. It is also used for hydrogen ion determinations and for titrations. The change in color occurring during a titration can be followed by the photocell and the apparatus can be so arranged that the amplified current or output of the cell can be made to interrupt the addition of the titrating agent at any desired endpoint.

This type of apparatus may also be used as a nephelometer or turbidometer for different turbidities will also cause variations in the photoelectric current. This factor is important and it is necessary to have no turbidity in all measurements depending on color for the photoelectric cell cannot distinguish the cause of reduction or increase in intensity of light, it merely measures that intensity.

Lovibond Tintometer—The Lovibond tintometer, used a great deal in England, is in reality a color comparator. The depth of color of

unknown solutions or materials is matched against the color of standard glasses. The instrument is simple, consisting of an elongated box with an eye piece at one end and two rectangular openings at the other, one for the solution or substance to be examined, the other for the standard glass slides used for matching the color. Solutions are placed in glass cells and are examined by transmitted light. Powders are pressed into a form and examined by reflected light.

The complete set of standard slides consists of 465 glasses, 155 each of red, yellow and blue. Each glass has a value expressed in an arbitrary Lovibond scale.

Red glasses transmit red, orange and violet
stop yellow, green and blue.
Yellow glasses transmit yellow, green and orange
stop red, blue and violet.
Blue glasses transmit green, blue and violet
stop red, orange and yellow.
Combinations of red and yellow glass transmit orange only.
Combinations of yellow and blue glass transmit green only.
Combinations of blue and red glass transmit violet only.
Combinations of all three transmit no color and only give a neutral tint.

Colorimeters of all types have many uses. In food chemistry determinations of nitrogen, phosphorus, lead, aluminium, citral, vanilla and sugar can be conveniently made colorimetrically. These methods will be detailed in other sections of the text. The selection of the proper colorimetric procedure depends on the method and the experience of the analyst. Thus where colors and tints are to be matched as in honey, beer, or vanilla extract, a Lovibond comparator or some similar device must be used. In lead spray determinations and in other determinations in the chapter on metals, methods using Nessler tubes advantageously are described. Where routine analyses are being made, as for example, nitrogen determinations, it is convenient to calibrate a curve on a photoelectric colorimeter and estimate the amount of nitrogen by that means. Where it is possible to obtain fairly uniform suspensions, a nephelometer may be used as in silver chloride determinations. In short, the instrument to use is the one which will give the best results for the determination and method involved.

The Munsell System—The standards for color in canned tomatoes under the McNary-Mapes Act [4] and for tomato juice, tomato pulp and tomato catsup formulated by the Bureau of Agricultural Economics [5] are all expressed in the Munsell System.[6,7] It is beyond the scope of this text to give a detailed consideration of the apparatus and technique necessary for the successful measurement of color by this system. However, an explanation of the notation used in the standards follows.

The definite valuation of the color of a particular product according to the Munsell System consists of two parts: The first is the percentage of the different specific colors which when blended together give a composite color which exactly matches the sample. The percentage notations of a particular color must always add up to 100 per cent. The second essential part of each color notation is the exact description of each of the color cards used to match the sample in terms of accurately determined values for "hue," "brilliance" and "chroma." Each class of agricultural product requires a particular set of color cards. For example, the color of canned tomatoes and tomato products may be matched by varying the proportions of four particular colors, namely, definite shades of red, yellow, neutral black and neutral gray. These colors are designated by specific formulae in each of which there is an empirical designation of first, hue; second, value; and third, chroma.

The formulae for the red color used in matching tomato products is 5R2.6/13. 5R indicates the *hue*, which is the attribute of color that permits colors to be classed as reddish, yellowish, greenish, or bluish. Five principal hues are used in the Munsell System, namely, red, yellow, green, blue and purple, designated R.Y.G.B. and P. Midway between these are five similar intermediate hues: yellow-red, green-yellow, etc., designated YR, GY, etc. The designation 5R is a pure red free from purple or yellow.

The second notation, "2.6" indicates the *value* or intensity of the black constituent of the color. This is expressed in an arbitrary scale from 0 to 10 where zero is absolute black and 10 is absolute white. A "value" of 2.6 indicates a considerable proportion of black constituent.

The final designation shows the *chroma*, which expresses the strength or intensity of the color. The notation of chroma is in an arbitrary scale

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[4] U. S. Dept. Agr., Food Drug Admin., S. R. No. 4, 4th rev. (1937).

[5] U. S. Dept. Agr., Bur. Agr. Econ. U. S. Standard for Grades of Canned Tomato Pulp and Tomato Catsup, January (1934).

[6] Cooper, "Munsell Manual of Color," Munsell Color Co. (1929).

[7] Nickerson, U. S. Dept. Agr., Bur. Agr. Econ., Tech. Bull. No. 154 (1928).

from 0 to 10 or even further, which in this case is 13, which indicates a very strong, intense red color.

The required color cards, which can be obtained only from the Munsell Color Company, are cut in the form of "Maxwell discs," which are uniform circles of each of the cards with a small hole at the center and a single radial slit from the center to the edge so that the discs may be placed one on the other and so slipped together by means of this radial slit so that any desired proportion of one or more of the color discs may be exposed as a segment of the single circle that is visible. When these discs are held together with a suitable binding post at the center and spun at a speed great enough to eliminate flicker, the resulting color seen by the eye is the sum of the different segments exposed. The discs should be slipped together in such a direction that the spinning will cause them to lie flat rather than to fly apart. The amount of each color card exposed is changed until the combined effect exactly matches the color of the sample. The amount of each color exposed is then measured by the per cent of the circumference occupied by each segment. The discs are mounted on a stiff card containing a permanent circular chart for measuring these percentages. So it is that the expression in the standard for color in tomato pulp "YR21 (2.5YR 5/12)" means that 21 per cent of the yellow-red disc is exposed and this yellow-red disc has the formula Hue 2.5YR, Value 5, Chroma 12.

It will be noted that the requirement for color of grade A tomato pulp according to the Bureau of Agricultural Economics is that it shall be "equal or better than that produced by spinning a combination of the following Munsell color discs. R65 (5R 2.6/13)-(glossy finish); YR21 (2.5YR 5/12)-(glossy finish); N1 (glossy finish); N4 (mat finish)." This means that when the color of the whirling color discs matches the color of the sample there must be at least 65 per cent of the red disc exposed and not more than 21 per cent of the yellow-red disc exposed and the remaining 14 per cent may be any combination of the black N1 and the gray N4. All of the color discs come in either the glossy finish or the mat finish so that it is necessary to specify which finish is desired when such color cards are being ordered.

Fluorescence Analysis – All substances absorb electromagnetic vibrations or light, usually over a characteristic range of wave-lengths, and many emit or re-emit such radiations. This emission phenomenon is known as luminescence. Luminescent light may be in the visible and

invisible regions. Luminescence may be exhibited by solids, liquids or gases and is classified under two sub-heads:

(1) Fluorescence, if the luminescence lasts only during the period of excitation,

(2) Phosphorescence, if the luminescence persists after the exciting source is removed.

When the luminescence produced is characteristic of the substance irradiated, it may be used as a means of analysis. For most cases it is sufficient to note the intensity and color and where greater specificity is desired, the light emitted may be examined spectroscopically or photometrically. In general, the intensity is proportional to the amount of active substance present consequently quantitative measurements may be made.

Radley and Grant [8] group fluorescent analysis methods as follows:

1) *Qualitative*

a) Direct Irradiation. The substance is placed in ultra-violet light, and the nature, color and intensity of the luminescence are noted and compared with the characteristics of that from genuine samples of known origin. This may be carried out in acid, alkaline or neutral solution, at various concentrations, and in a number of solvents.

b) A refinement of the above is to observe the luminescence under a luminescence-microscope or to determine its spectral characteristics.

c) Chemical Reactions. The suspected substance is treated with a chemical reagent which should produce a luminescent compound, and the appearance of such a compound observed. Conversely, if the substance is itself luminescent, a reagent may be chosen which destroys the luminescence of the substance concerned.

d) Capillary Analysis. If a filter paper is held vertically with one edge in the solution, it draws up the liquid by capillary attraction, and when the wet portion is examined under the lamp, characteristic zones are obtained from many substances.

2) *Quantitative.*

a) Trial and Error. A number of mixtures containing known quantities of the luminescent substance are compared with the sample in

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[8] Radley and Grant, "Fluorescence Analysis," Van Nostrand (1933).

ultra-violet light, and an approximate match is obtained with one of them.

b) Photometry. The intensity of the luminescence, which, other conditions being equal, is proportional to the amount of luminescent substance present, may be determined photometrically.

c) Luminescent Indicators. Determinations of pH may be made by means of indicators whose luminescence in ultra-violet light changes with change in hydrogen ion concentration. These, and similar compounds, may be used to indicate end-points of neutralization or oxidation-reduction titrations, and are usually sensitive in very small quantities and in very great dilutions of reagent, as well as being free from many of the usual errors inherent in the use of indicators. Thus Grant [9] points out the use of quinine sulfate as a fluorescent indicator for precipitation reactions. Its action appears to depend on the fact that quinine sulfate fluoresces in the presence of an excess of some ions but not of others, so that if the end-point of the reaction involves a change from one to another of these types of ions it will be indicated by the production or disappearance of fluorescence.

The titration should take place in a thin-walled 200 cc. conical flask, which is supported on a black base in such a position that the filtered ultra-violet light falls on it from the side; it is preferable to arrange the lamp so that it is slightly above the level of the liquid in the flask. The solution in the flask should be as concentrated as is conveniently possible, and it should be shaken well after each addition of reagent to stimulate coagulation, as this leaves a supernatant liquid in which the fluorescence can be observed. Only a small pinch of solid quinine sulfate need be used, and a darkened room is not essential if colorless 0.1 *N* solutions are being titrated. Titrating sodium chloride against silver nitrate, for example, a change from bright pale blue to dull purple is obtained at the end point.

If solvents are used in this type of analysis, they should be optically inert and non-fluorescent. Ether, petroleum ether, amyl alcohol and chloroform are often used. The materials should be examined in dishes of non-fluorescent glass or porcelain. Containers made from black filter paper are effective for solids which give a white fluorescence. Petri dishes covered with Cellophane are useful in keeping out contamination.

Fluorescent analysis is a newer form of analytical procedure, never-

[9] Grant, *Analyst* 62, 285 (1937).

theless, a large amount of work has been done on foods. These will be referred to in the appropriate sections of the text.

Spectrometry

When a parallel beam of light passes through a prism, it is refracted and dispersed because the index of refraction of glass is greater for violet than for red light. Such dispersed bands of light are called spectra. If the light coming from an incandescent substance is passed through a prism, a spectrum characteristic of that substance is formed. Hence by observing the emission spectrum, that is a flame, arc, spark or other spectrum of an unknown material, its nature may be ascertained from the definite lines and bands its spectrum forms.

Fig. 19. Spectrometer (Courtesy of Bausch & Lomb)

Emission spectra can be produced in a number of ways. When a more or less volatile metallic salt is placed in contact with a flame and is subsequently volatilized by the flame, flame spectra are produced. A convenient method is to place the salt in solution and then feed it into the flame by means of a platinum wick. Arc spectra may be produced from substances that are not easily fusible. Rods of the metal may serve as the poles of the arc or the metal may serve as the negative pole and carbon as the positive pole. Alternative means of producing arc spectra are to use rods of carbon having a central core of the metal or placing small pieces of the substance in a crater of the positive pole of the carbon rod. Spark spectra are produced by means of sparks from an induction coil made to pass between small poles of the substance or discharged through gases or passed between a platinum wire and a solution of a salt of a metal. Another form is the use of cathode streams for the production of phosphorescence in solid substances.

If white or other light is passed through an absorbing medium before being passed through the prism, a dark band will appear in the spectrum and will be characteristic of the absorbing medium. Such a spectrum is called an absorption spectrum.

The spectrometer, Fig. 19, consists of a collimator which is a tube having a narrow slit and lens to provide a parallel ray of light, a prism, to refract the light and form the spectrum, a telescope to view the spectrum and a third tube which has a scale that may be reflected into the ocular and so be used for calibrating the spectrum. This instrument is called an angular vision spectrometer.

The principle of the direct vision spectrometer, Fig. 20, is to make the dispersed light emerge from the same direction from which it came

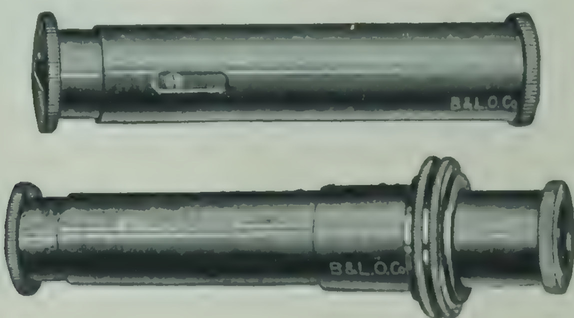


FIG. 20. Direct Vision Spectrometers. (Courtesy of Bausch & Lomb)

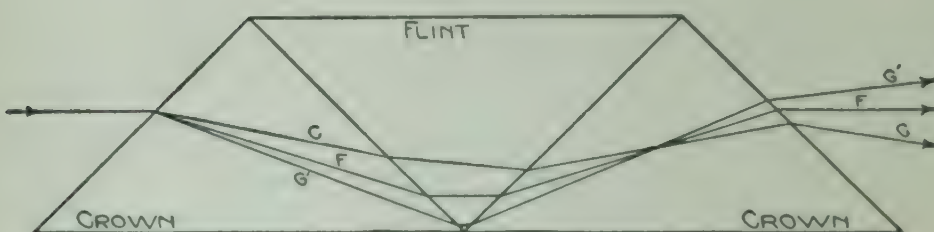


FIG. 21. Amici Prism. (Courtesy of Bausch & Lomb)

by means of a suitable system of prisms, as for example, the Amici prism, Fig. 21. A small direct vision spectrometer screwed into position instead of the eye piece of a colorimeter, as for example, a Klett, can be used to compare the absorption spectra of two solutions.

The spectrometer is used, generally, as a qualitative instrument in contradistinction to the colorimeter and polarimeter. There are, however, newer, spectrographs and photospectrometers, Fig. 22 (spectrometers equipped with photographic apparatus) which are quantitative instruments. Essentially a spectrophotometer is a device for determining wave length by wave length the proportion of radiant energy incident

upon a body that is reflected or transmitted by it. Whereas a colorimeter is limited to colored solutions, the spectrophotometer is not limited to colored solutions, but can be used for ultraviolet or infra red spectra and for both opaque and transparent substances.

All methods of quantitative spectrum analysis are based on the fact that, the greater the percentage of a metal in a sample, the stronger will be the spectrum lines of that metal, that is, the concentration of the metal varies directly with the intensity. A useful method is that of Lewis [10] and is based on the principle of synthesizing a specimen so that its spectrum exactly matches that of the sample.

It is necessary to bring the whole sample of ash or other material

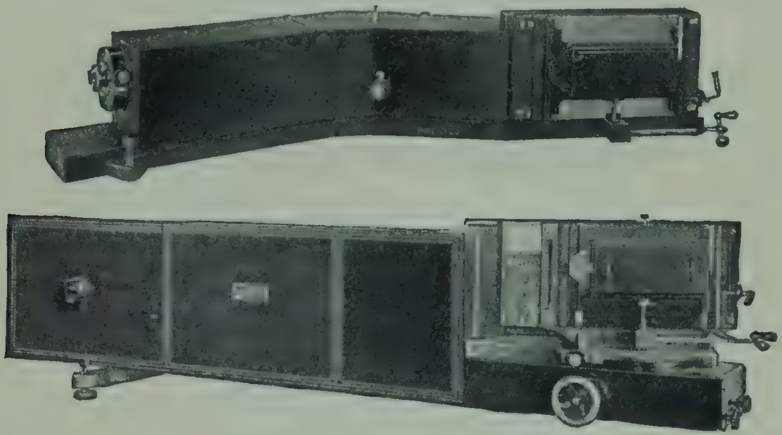


FIG. 22. Spectrograph. (Courtesy of Bausch & Lomb)

into some constant homogeneous condition and to arc or spark under well defined uniform control. This may be done by treating the ash including any phosphate or silicate or other insoluble matter with a small excess of sulfuric acid. The mixture is evaporated and ignited to drive off excess sulfuric acid. The mass may now be assumed to consist mainly of normal sulfates. It is then mixed with an equal amount of ammonium sulfate, $(NH_4)_2SO_4$, to regularize its action while burnt in the arc and is ready for spectrographing.

The first spectrogram will reveal the approximate composition from the intensity of the metal lines as stated above. If precise results are desired a special ratio powder may now be prepared in accordance with the findings and the spectrographing repeated.

[10] Lewis, *Analyst* 60, 11 (1935).

Absorption spectrograms are discussed in some detail in the section "Spectrographic Method" for vitamin A in the chapter on Vitamins.

These instruments are used in food analysis to detect metallic contamination, to distinguish dyes both natural and artificial, to detect the presence of foreign oils in olive oil and for general absorption determinations.

Polarimetry

When light is propagated, the wave front of the light is in motion in all directions. If this ordinary light is passed through a Nicol prism, a crystal of Iceland spar, the vibrations of the emergent ray take place

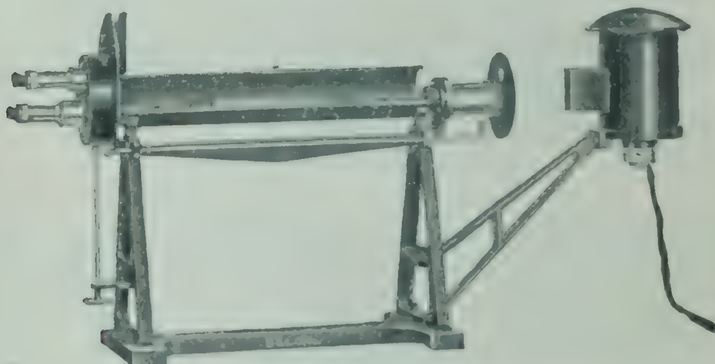


Fig. 23. Polarimeter. (Courtesy of Bausch & Lomb)

in one plane and the ray is said to be plane polarized. If such a ray of plane polarized light is passed through a solution of a substance or through a crystal of a substance which is molecularly asymmetric, the polarized ray will be turned to the right or left. Organic substances in whose molecules, four different groups are attached to a carbon atom are asymmetric and will rotate a polarized ray. Many other substances both inorganic and organic having different groups arranged around an atom or in which the molecule as a whole is asymmetric will also turn a polarized ray. Substances which rotate the ray of polarized light to the right are called dextrorotatory and those which rotate it to the left are termed laevorotatory.

The polarimeter, Fig. 23, is an instrument which measures the angle through which the polarized ray is turned to the right or left. The instrument consists of a slit and lens to make the rays from a source of

monochromatic light parallel, a polarizer or fixed Nicol prism to make the light vibrate in one plane, a tube to contain the rotating solution, an analyzer prism which can be turned, a system of lenses to observe the rotation and a scale by which the angle of rotation is measured. The tube, Figs. 24 and 25, which is of definite length is filled with the unknown solution. It is placed in position and then the analyzer is

Fig. 24. Polarimeter Tube

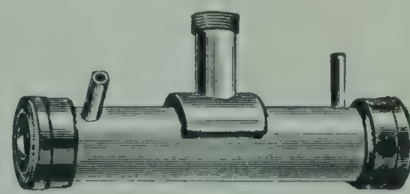


Fig. 25. Polarimeter Tube, Jacketed

(From Thurston's Pharmaceutical and Food Analysis. D. Van Nostrand Co., Inc.)

turned to maximum transmission of light or complete extinction. The rotation is given in degrees. The specific rotation $[a]$ is given by

$$[a] = \frac{100a}{lc}$$

where

$a =$ the rotation observed in degrees
$l =$ the length of the tube in decimeters
$c =$ the concentration of the active substance in grams per 100 cc.

The molecular (or equivalent) rotation is usually given as:

$$[a_M] = \frac{M[a]}{100} = \frac{Ma}{lc}$$

where

$M =$ The molecular (or equivalent) weight of the active substance.

$[a_M]$ is thus the rotation per mole (or per equivalent) per 1 cc. of solution for a column 1 mm. long, or per mole (or per equivalent) per liter of solution for a column 1 meter long. The rotation is usually given for monochromatic light, generally the sodium line or one of the mercury lines. If the polarimeter scale is calibrated to read the arbitrary units of the sugar scales of Ventzke, or Bureau of Standards, etc., it is called a saccharimeter. The tube generally used is termed a normal

tube and is exactly 2.0 dm. long. At times depending on high rotation, on depth of color and other factors, a half-normal or 1.0 dm. tube is used. For low rotation, a double normal or 4.0 dm. tube is used. For readings at higher temperatures, for example, 87° C., a jacketed tube is used. The polarimeter in food analysis is used mainly for the estimation of sugar solutions and essential oils.

Electrometric Determinations

Electrometric determinations are so often concerned with the estimation of hydrogen ion concentration, that a brief discussion is appropriate. Clark [11] suggested that it is convenient to consider the term normality as the quantity factor of acidity or alkalinity and the hydrogen ion concentration as the intensity factor. Hydrogen ion concentrations vary ordinarily from 1 g. of dissociated ions per liter to less than 1 million-millionth. These concentrations are expressed as:

$$
\begin{aligned}
1 &= 1 \times 10^{0} \\
0.1 &= 1 \times 10^{-1} \\
0.01 &= 1 \times 10^{-2} \\
0.000001 &= 1 \times 10^{-6} \qquad \text{etc.}
\end{aligned}
$$

Rarely, however, is the concentration an even decimal fraction. Other concentrations may also be written in the same notation, namely,

$$0.000472 \text{ g. per liter} = 4.72 \times 10^{-4} \text{ g. per liter.}$$

Sørensen and Clark introduced a simpler means of notation. Since a logarithm is an exponent of the power of 10, the concentration can be stated in terms of the logarithm, thus:

$$[H^+] = 1 \times 10^{-4} \text{ can be written } \log [H^+] = -4 \text{ or } -\log [H^+] = 4$$

and since a negative logarithm indicates that the quantity is a fraction, the concentration can be expressed as the reciprocal of $[H^+]$, that is, $\log \frac{1}{[H^+]} = 4$. Clark assumed that a symbol

$$\text{pH} = \log \frac{1}{[H^+]} \text{ or } [H^+] = 10^{-\text{pH}},$$

hence, in the above example, pH = 4. The pH value of a solution is then the logarithm of the reciprocal of the hydrogen ion concentration.

[11] Clark, "Determination of Hydrogen Ion," Williams and Wilkins (1920).

Exponential expressions of hydrogen ion concentration can be converted to pH expressions and vice versa. For example, a solution of acetic acid has a hydrogen ion concentration equal to 1.36×10^{-3} g. per liter. The corresponding pH may be found as follows:

$$\begin{aligned} pH &= \log \frac{1}{[H^+]} \\ &= \log \frac{1}{1.36 \times 10^{-3}} = \log \frac{10^3}{1.36} \\ &= \log 10^3 - \log 1.36 \\ &= 3.00000 - 0.13354 \\ &= 2.86646 \end{aligned}$$

or as more usually expressed, to the nearest hundredth, pH = 2.87.

For the converse example, let us assume a solution of ammonium hydroxide has a pH = 10.77. The hydrogen ion concentration may be ascertained as follows:

$$pH = 10.77 \text{ is equivalent to } [H^+] = 1 \times 10^{-10.77}$$

The exponent $-10.77 = \overline{11}.000 + 0.230$. These numbers are logarithms, and looking up the antilogarithms, that is numbers whose logarithms have the given value, we find the antilogarithm of 0.230 is 1.7, therefore:

$$[H^+] = 1.7 \times 10^{-11}.$$

On the pH scale, the hydrogen ion concentration of pure water is 7 at 22° C., while a normal solution of hydrochloric acid approaches 0 pH and a normal solution of sodium hydroxide approaches 14 pH.

Measurement of pH in solutions in common use show results widely different from those with pure solutions, because of the effect of certain substances in the solution on the dissociation of acid and base molecules. When a neutralizing reagent is added to an acid solution containing substances which repress dissociation, the change in pH is less than that occurring when the same quantity of reagent is added to the pure acid solution. This effect is called the buffer action of the substances other than the acid. The power of certain solutions to resist change in hydrogen ion or hydroxyl ion concentration on the addition of acids or alkalis, is known as buffer action and such solutions are known as buffer solutions.

TABLE 2. COMPARISON OF pH SCALE WITH H-ION CONCENTRATION

		strong acid						pure water							strong base	
pH scale	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
$[H^+]$ in g/liter	$\frac{1}{1}$	$\frac{1}{10}$	$\frac{1}{100}$	$\frac{1}{1000}$			$\frac{1}{1000000}$			$\frac{1}{1000000000}$			$\frac{1}{1000000000000}$			fractional notation
	10^0	10^{-1}		10^{-3}			10^{-6}			10^{-9}			10^{-12}			exponential notation

Potentiometer—A potentiometer, Fig. 26, is a device for measuring potential differences by either totally or partially balancing the unknown e. m. f. against a variable potential difference, the value of which is known by reference to a standard of electromotive force. If the two potential differences are exactly balanced, we have the usual "null" potentiometer, if partially balanced, the galvanometer gives a measure of their difference and we have a deflection potentiometer. This instrument, Fig. 26, consists essentially of a source of current generally a 2 volt storage battery or two 1 volt dry cells, a slide wire arrangement

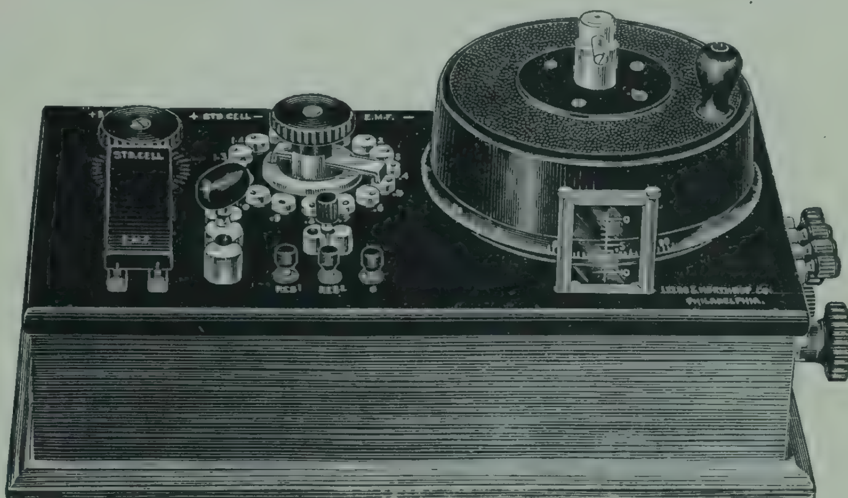


FIG. 26. Potentiometer. (Courtesy of Leeds and Northrup)

such as a Leeds and Northrup potentiometer, a slide rheostat to control the fall of potential along the slide wire and act as a reference potential, a galvanometer to show the direction of current flow and an unknown solution which is turned into a cell with the aid of electrodes. If E in Fig. 27, represents the cell for hydrogen ion measurements the calomel electrode is the positive pole P, which is connected to M, and the hydrogen electrode is the negative pole N, which is connected to M^1 through the galvanometer G. This puts like polarities in opposition. By adjusting the position of M and M^1 the potential difference between them can be made exactly equal to that between P and N. When the voltages are balanced the deflection of the galvanometer is zero, and since this is a current-indicating instrument, it proves that no current is flowing be-

tween the electrodes. Then if the fall of the potential between is known, the voltage of D is measured without any flow of current from E.

The fall of potential between M and M[1] is determined when there

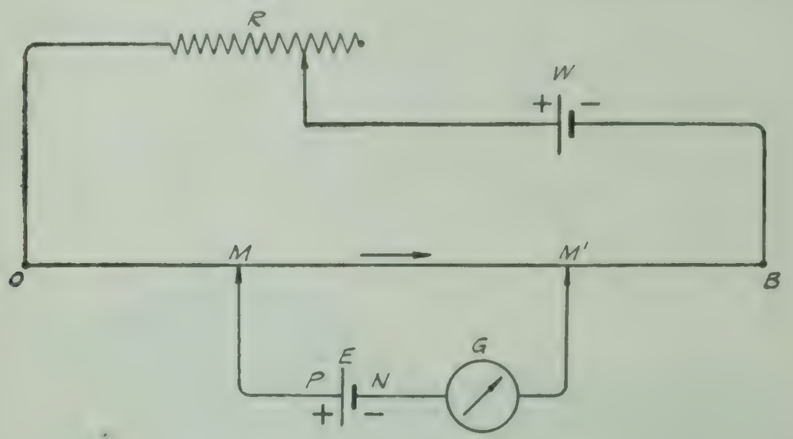


FIG. 27. Potentiometer Diagram
(Courtesy of Leeds and Northrup)

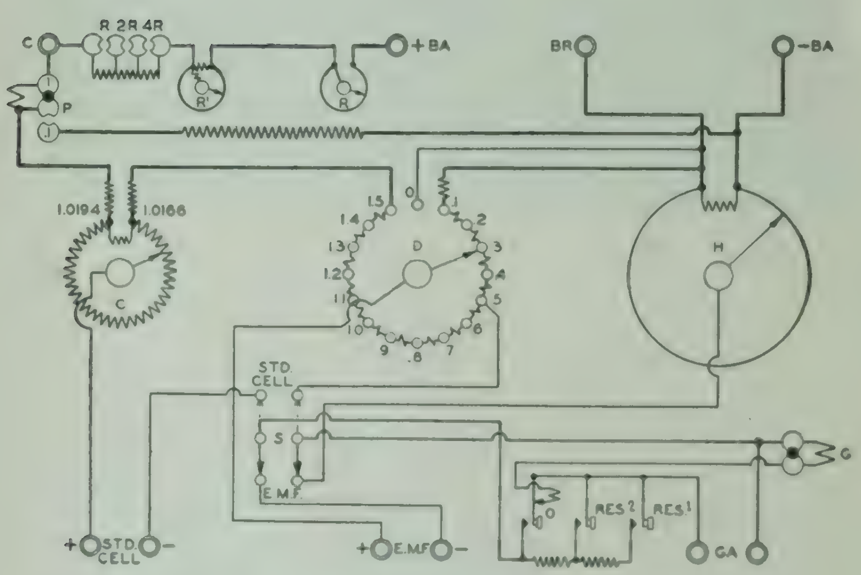


FIG. 28. Potentiometer Diagram, Detailed
(Courtesy of Leeds and Northrup)

is a known potential difference between O and B. This is established by replacing the hydrogen ion cell with a standard cell having its electrodes connected according to the polarity indicated. If the potential

difference between its electrodes is, for example, 1.0183 volts, the contacts M and M[1] are set to span 1018.3 divisions of the scale, and the current in the calibrated resistance OB is adjusted by means of the rheostat R until the galvanometer shows no deflection. The potential difference between M and M[1] is then 1.0183 volts, and the fall of potential for each scale division is 0.001 volt or 1 millivolt. If the hydrogen ion cell is now substituted for the standard cell, the potential difference between its electrodes will be shown directly in millivolts by the number of scale divisions between M and M[1] when they are now adjusted for voltage balance.

The unknown solution may be set up as a cell by the use of a saturated

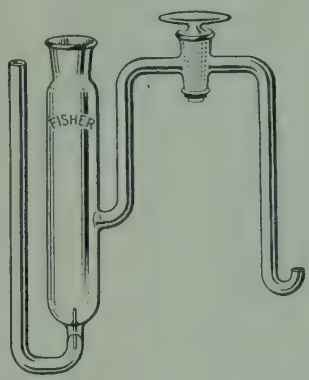


Fig. 29. Calomel Half-Cell

(Courtesy of Fisher Scientific)

Fig. 30. Hydrogen Electrode

(Courtesy of Fisher Scientific)

calomel half-cell, Fig. 29, as the reference electrode and a hydrogen electrode, Fig. 30, as in Fig. 31. The electromotive force of this cell is measured with the aid of the potentiometer and the hydrogen ion concentration or pH of the solution is ascertained by the formula:

$$\mathrm{pH} = \frac{V - 0.245}{0.0591} \qquad \text{at } 25^\circ \text{ C.}$$

where

V = the observed potential.

While the hydrogen electrode is of high accuracy and is applicable to the entire pH range, it is not applicable in the presence of dissolved

gases, foaming solutions, oxidizing or reducing solutions, and is easily poisoned by hydrogen sulfide, alkaloids and hydrocarbons.

Some of the difficulties encountered in the use of the hydrogen electrode are avoided by using a quinhydrone electrode. Quinhydrone is an equimolecular compound of quinone and hydroquinone. It is only

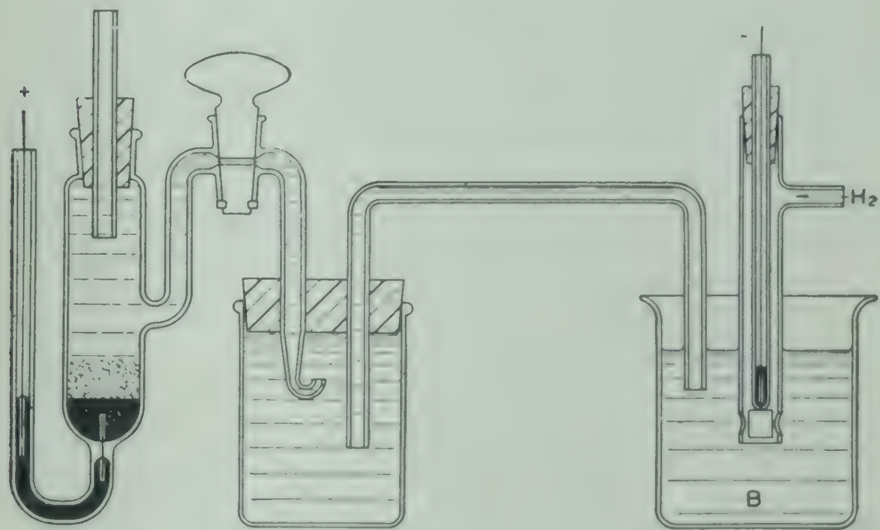


FIG. 31. Cell. (Courtesy of Leeds and Northrup)

slightly soluble in water, as only 3.94 g. are present in a liter of solution at 25° C., but it is 93 per cent dissociated to quinone and hydroquinone.

$$(C_6H_4)_2O_2(OH)_2 \rightleftarrows C_6H_4O_2 + C_6H_4(OH)_2$$

The probable transformations which occur are:

quinone + 2ε ⇄ anion of hydroquinone, and
anion of hydroquinone + $2H^+$ ⇄ hydroquinone, or alternatively expressed

$$QH_2 \rightleftarrows Q + 2H^+ + 2\varepsilon$$

The quinhydrone half-cell is very simple. It consists of a metal such as platinum or gold as the electrode and a certain amount of quinhydrone is added to a given volume of solution. This half cell may then be placed in combination with a saturated calomel electrode and the difference in

e. m. f. determined by means of the potentiometer. In this case, the hydrogen ion concentration or pH of a solution is given by the equation:

$$pH = \frac{0.453 - V}{0.0591} \qquad \text{at } 25^\circ \text{ C.}$$

where

$V =$ the observed potential.

The advantage of the quinhydrone electrode is that it is simple and

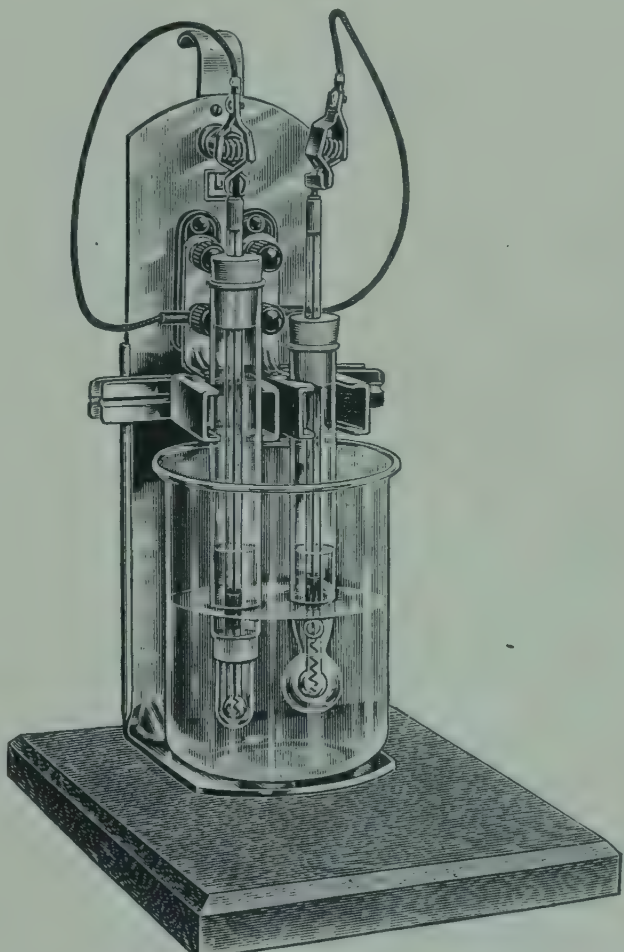


FIG. 32. Glass Electrode Cell. (Courtesy of Leeds and Northrup)

needs no catalytic metal surface or gas. Its disadvantage is that it cannot be used for determination of pH greater than 9.

The glass electrode consists of a glass membrane, Fig. 32, and the

potential difference set up at the two sides of the membrane is measured through the use of an internal and external electrode system. The glass membrane is made of soda lime glass. The e. m. f. of the cell varies linearly with the hydrogen ion activity over a considerable range of concentration. A high sensitivity galvanometer or a thermionic amplifier must be used in conjunction with the glass electrode because of its high resistance.

The glass electrode may be used to measure oxidation-reduction systems and is the only pH electrode adaptable to non-buffer solutions. Its disadvantages are that it is easily broken and that it has a high resistance.

The potentiometer is not limited to the measurement of a single pH but can be used too for making routine titrations and for many procedures in which a potentiometric end point can be obtained. Thus in following a titration, the e. m. f. may be measured upon successive small additions of acid or alkali in a titration. The pH values derived are then plotted as ordinates and the ccs. of reagent as abscissae. First, there is a more or less gradual slope due to the slowly increasing effect of the base upon the acid, then a decided bend as the quantity of base becomes more nearly equivalent to that of the acid, and finally a precipitous drop in the curve with the addition of a very slight amount of alkali, indicating that the reaction has passed the neutral point. With further addition of base, the curve bends again but in the opposite direction, and returns to a gradual slope as the solution becomes increasingly alkaline.

In making routine titrations it is not necessary to develop a complete curve. The potentiometer can be set at the hydrogen ion concentration to which the reaction is to be carried, and the reagent allowed to flow into the solution quite rapidly, the key closing the galvanometer circuit being tapped frequently to observe the progress of the neutralization. At the beginning, the deflections will be lively, but they will become less pronounced as the solution approaches the neutral condition. Just before the deflection becomes zero the stop cock is closed, the burette is read, and the solution thoroughly stirred. The reagent is now added slowly until the equivalent point is reached, at which point the addition of a single drop of reagent will cause a large change in the concentration of hydrogen ions, as will be observed on resetting the potentiometer.

The potentiometer can be used for many reactions in which a difference of potential can be obtained. Many of these methods are applicable to

the analysis of foodstuffs. The simple instrument is being replaced in order to do away with reference electrodes by the use of mono-metallic polarized electrodes, the bi-metallic platinum-tungsten system and electronic methods using a continuous reading micro-ammeter. One of the newer potentiometric devices uses a 6E5 cathode ray tube, the "magic eye" visual tuning indicator of radio, and merely requires plugging into the usual 110 volt alternating current line. The titrating solution is added as usual from a burette and the end point is noted by the non-fluorescent sector of the screen attached to the apparatus opening a full 90°.

In the food laboratory, the potentiometer is used for the determination of the hydrogen ion concentration of materials such as milk, other dairy products, plant and fruit juices, for following titrations and in general when accurate measurements of acidity and alkalinity need be made. For example, Mitchell[12] made a study of the pH of tomato juice, using the glass, quinhydrone and hydrogen electrodes for comparison.

Conductometric Measurements—The conductivity of an electrolyte is not measured directly, but is estimated from a measurement of the resistance of the solution between two electrodes immersed in it. The resistance offered by a 1 cm. cube of a conductor is termed the specific resistance or resistivity. The reciprocal of this is called the specific conductance or conductivity and is denoted κ. Equivalent conductivity is the conductance of a solution containing 1 gram-equivalent of a solute placed between 2 electrodes of indefinite size and 1 cm. apart and is denoted Λ. It is equal to the conductivity κ multiplied by the volume in ccs. [ϕ] containing 1 gram-equivalent of solute, that is

$$\Lambda = \kappa\phi.$$

The resistivity or conversely the conductivity of a solution is generally measured by means of a Wheatstone bridge. The four resistances of this system are so arranged that if three of them are known, the fourth may be calculated. A conductivity apparatus, Fig. 50, has a cell to contain the unknown solution which acts as the unknown resistance. This cell is connected in series with a known but variable resistance. The other two arms of the bridge consist of a slide wire of uniform resistivity

12 Mitchell, *J. Assoc. Official Agr. Chem.* **18**, 128 (1935).

with a sliding contact to provide a known ratio. An alternating current and a telephone or an equivalent device to tell the point of minimum sound or current and thus the equality of the ratios completes the set-up. To make a measurement, the sliding contact is moved until the sound in the telephone is a minimum. Since the variable resistance is known and the slide wire ratio may be read, the unknown resistance is given by:

$$\frac{X}{R} = \frac{A}{1000 - A}$$

and

$$X = \frac{A}{1000 - A} R$$

in which

X = the resistance furnished by the unknown in its cell

R = the variable known resistance

$\frac{A}{1000 - A}$ = the variable ratio obtained from the slide wire which is divided into 1000 parts.

Zerban and Sattler [13] and other investigators have used the conductometric method to determine the ash content in raw cane sugar, refined cane sugar and allied sugar products. The principle of the method is that sugar is a non-conductor and that only the electrolytes, which are present in the sugar will conduct. Hence a relationship exists between the conductivity value of a sugar and its ash. The conductivity value of maple products is an official A. O. A. C. method. Conlin [14] describes a method which is given in detail in the chapter on sugars.

Conductivity measurements may be used to follow usual titrations, neutralizations, precipitation reactions and, in general, where a minimum in conductivity may be obtained. In fact, whenever a reaction between substances dissolved in two liquids produces such changes in the number or mobility of the ions in the first liquid as to cause its conductivity to reach a maximum or minimum at the completion of the reaction, the end point can be conveniently determined by following the conductivity changes occurring in the solution. Thus whether the solutions are very

[13] Zerban and Sattler, *Ind. Eng. Chem., Anal. Ed.* 3, 41 (1931).
[14] Conlin, *Ind. Eng. Chem., Anal. Ed.* 7, 426 (1935).

highly colored or very dilute, reactions taking place within them are still susceptible to this type of measurement.

If then conductance values or more simply bridge values $\left[\frac{A}{1000 - A}\right]$ are plotted as ordinates against cc. of reagent added as abscissae, a titration graph is obtained which consists of two intersecting straight lines. It is obvious, that due to the withdrawal of hydrogen ions and hydroxyl ions or other reacting ions as the neutralization or precipitation proceeds, the conductivity will reach a minimum and then will increase on the further addition of the titrating agent. The point of minimum conductivity is the end point and is obtained graphically.

Viscosimeter

The viscosity of a fluid is the internal friction which tends to bring to rest portions of the fluid which are moving relative to one another. It is the resistance which particles of a fluid offer to free motion one over the other. It is measured in relation to some standard viscosity generally water at 25° C. or sugar solutions. Liquids which do not have a high viscosity may be measured by determining the time rate of flow through a capillary of a definite volume of the fluid and then applying Poiseuille's formula:

$$\eta = \frac{\pi p r^4 t}{8Vl}$$

in which

η = coefficient of viscosity
p = the driving force
r = the radius of the capillary tube
t = the time required for
V = the volume of liquid to flow through the tube
l = length

Fluids which have a high viscosity cannot be measured by such means and other devices are used. The Engler and Saybolt viscosimeters use the principle of measuring the time rate of flow of a given quantity of material, as for example, oil through a standard orifice.

The MacMichael, Fig. 33, and the Doolittle viscosimeters operate on the torsion principle. A plunger of standard dimensions is suspended by a torsion wire of exact length from the top of the instrument. The

material is placed in a cup, which is revolved at a constant rate of speed on a motor driven platform mounted on ball bearing. The amount of twist imparted to the wire, depending upon the viscosity of the material, is read on a graduated disc attached to the spindle. The readings are in arbitrary degrees *M* (1/300 circle). However, by standardizing against solutions of known viscosity in poises, the results can be interpreted in poises, *i.e.*, C. G. S. absolute units of viscosity.

The viscosities of oils, gum solutions, gelatin solutions, ice cream mixes, sugar solutions, acidulated flour, serums and like materials and prepared solutions and mixtures can be obtained by the use of this instrument and similar instruments and are valuable indices of whether a food material is standard or substandard.[15]

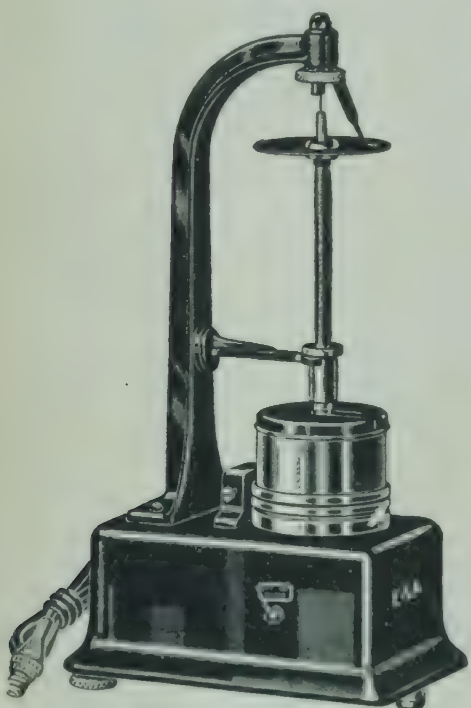


FIG. 33. MacMichael Viscosimeter (Courtesy of Eimer & Amend)

SURFACE TENSION APPARATUS

The surface particles on any given liquid have greater molecular forces tending to pull these particles toward the interior of the liquid than outward. Hence, a fluid surface acts like a stretched elastic membrane. This effect is called surface tension. It measures the work bringing molecules from the interior to the surface of the liquid and numerically is the force necessary to balance the tendency to contract in a strip of surface film one centimeter in width. It is obvious that the surface tension of a substance is characteristic of that substance. Surface tension is measured in dynes per centimeter.

There are two classes of methods of measurements for surface tension, the static and dynamic. Among the static methods are the capillary rise, weight of hanging drops, adhesion of a disk to a surface and direct measurement of curvature of the surface at contact with a plane. Among

[15] Harrel, *J. Assoc. Official Agr. Chem.* 18, 577 (1935).

the dynamic methods are oscillating jet, capillary undulations, etc., in which new surfaces are being formed. An instrument designed to measure surface tension easily, rapidly and accurately is that of Du Noüy. This apparatus is simply a torsion balance, which instead of measuring the tension by means of weights, uses the torsion of the wire to counteract the tension of the liquid film and break it. A single reading on a dial indicating the degree of torsion of the wire gives a figure, which,

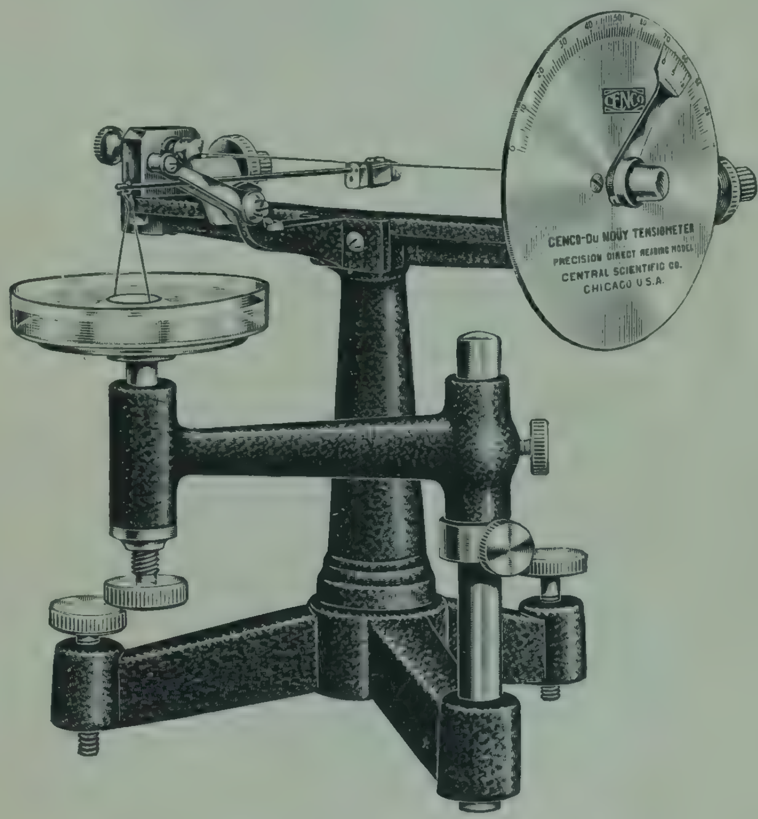


FIG. 34. Du Noüy Surface Tension Apparatus
(Courtesy of Central Scientific Company)

if the apparatus has been previously standardized with water, gives the surface tension of the liquid by a simple proportion.

The instrument, Fig. 34, consists essentially of a stand provided at the top with a fine steel wire stretched between end supports. One end of the wire is tightly clamped, the other being attached to a worm wheel controlled by a thumb-screw. To the worm wheel is also attached a pointer which moves over a metal scale graduated in degrees. To the

middle of the wire is clamped a hollow, light steel lever with a small hook in the outer end. A stirrup is attached to this hook carrying a carefully made loop of platinum iridium wire with a periphery exactly 4 cm. in length.

The liquid in a watch glass is placed on the platform and carefully raised by means of the adjusting screw until the platinum loop has made contact with the liquid. The pointer having been previously set at zero, the torsion of the wire is gradually increased by means of the thumb-screw controlling the worm gear, until the loop of wire tears loose from the liquid. The number of degrees is then read from the scale and by a simple calculation is converted directly into dynes per centimeter.

Another method of measuring surface tension is to determine the drop-number by means of a Traube stalagmometer, to ascertain the density and then calculate the surface tension.

Surface tension methods are valuable for indicating the purity of oils and alcohols. Instruments of this type have been used to determine constants of serums such as those made from milk.

Freezing Point Determinations

A property depending entirely on the number of molecules present is termed a colligative property. Such colligative properties are the volume of a gas and for substances in solution, vapor pressure, osmotic pressure, elevation of boiling point, depression of freezing point and others. In food analysis, we seldom use methods that estimate any of these colligative properties other than that of freezing point depressions. Van't Hoff showed that these properties were all interrelated.

Since the freezing point depression is dependent on the number of dissolved particles, then quantities proportional to the molecular weights, that is quantities containing the same number of molecules, dissolved in identical weights of a solvent, will cause equal depressions of the freezing point of that solvent.

It is known that the osmotic pressure of body fluids, such as blood and milk are almost constant. Hence the freezing points of these fluids is also almost constant. This constant freezing point in milk is due to the combined effects of three of the soluble components of milk, namely, lactose, the salts of potassium and sodium and the salts of calcium and magnesium. The smallest osmotic pressure of genuine milk ever observed is represented by a freezing point depression of about 0.53° C., as determined by the Hortvet method. The average depression obtained

by different observers varies from 0.54° to 0.55° C. If, therefore, a fresh milk gives a freezing point depression of less than 0.53° C., it is definite proof of the presence of added water.[16]

Thus from an estimation of the freezing point of a milk the amount of added water may be ascertained. The method in general use, an official

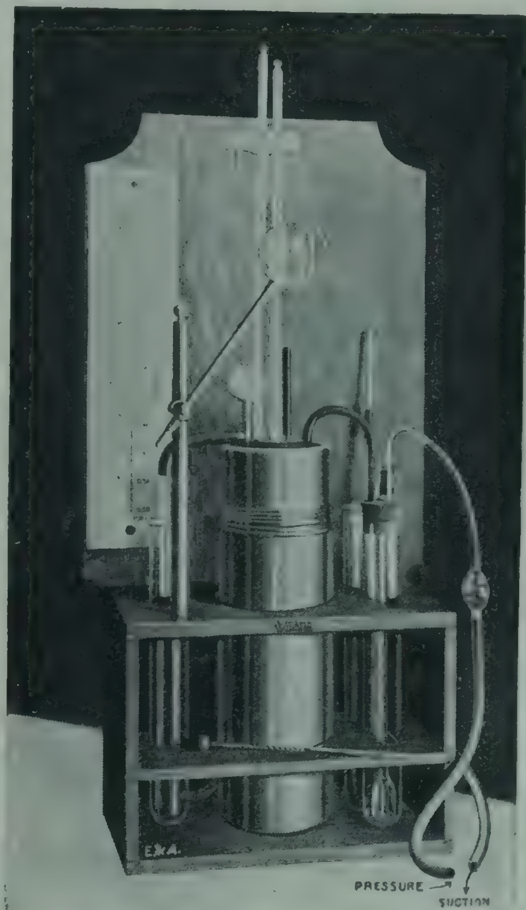


FIG. 35. Freezing Point Apparatus
(Courtesy of Eimer & Amend)

method of the A. O. A. C., is that of Hortvet.[17] The apparatus, Fig. 35, consists of an inner glass tube for the sample; a larger metal tube to hold the glass tube; a vacuum flask to insure constant temperature conditions; a metal stirrer; a metal tube for vaporizing the solvent producing the low temperatures; a metal "T" shaped tube; a large cork stopper;

[16] Evans, *Analyst* 61, 666 (1936).
[17] Hortvet, *Ind. Eng. Chem.* 13, 198 (1921).

an inner and outer air drying tube; a glass tube for holding thermometers when not in use; a freezing starter; glass funnel for pouring in the solvent; a vapor disposal tube; a special thermometer graduated from 1° above zero C. to 2° below zero, subdivided into 0.01° C.; a control thermometer graduated from 30° above zero C. to 30° below zero, subdivided in 1/1° C.; a thermometer scale magnifier for observing accurately and estimating to 0.001° C.; a small cork mallet for tapping thermometer; and a supporting stand including a milk glass scale.

Determination. Insert a small caliber funnel tube into the vertical portion of the "T" tube at one side of the apparatus and pour in 400 cc. of ether previously cooled to 10° C. or lower. Close the vertical tube by means of a small cork and connect the pressure pump to the inlet tube of the air drying attachment. Adjust the pump so as to pass air through the apparatus at a moderate rate, as may be judged by the agitation of the sulfuric acid in the drying tube. Continuous vaporization of the ether will cause a lowering of the temperature in the flask, from ordinary room temperature to 0° C. in about 8 minutes. Continue the temperature lowering until the control thermometer registers near −3° C. At this stage, by lowering a narrow gauge graduated glass tube into the ether bath, then closing the top by means of the forefinger and raising to a suitable height, an estimate can be made as to the amount of ether necessary to pour in for the purpose of restoring the 400 cc. volume. When the apparatus has once been cooled down to the proper temperature, an additional 10 to 15 cc. of ether is sufficient on an average for each succeeding determination. Measure into the freezing test tube 30 to 35 cc. of boiled distilled water, cooled to 10° C. or lower. Enough water should be measured in to fairly submerge the thermometer bulb. Insert the thermometer together with the stirrer and lower the test tube into the larger tube. A small quantity of alcohol, sufficient to fill the space between the two test tubes, will serve to complete the conducting medium between the interior of the apparatus and the liquid to be tested. A sufficiently tight connection between the inner and outer tubes is afforded by means of a narrow section of thin walled rubber tubing. Keep the stirrer in steady up and down motion at a rate of approximately one stroke each two or three seconds, or even at a slower rate providing the cooling proceeds satisfactorily. Maintain passage of air through the apparatus until the temperature of the cooling bath reaches −2.5° C., at which time the top of the mercury thread in the standard thermometer usually recedes to a position in the neighborhood of the probable freezing point of water. Maintain the temperature of the cooling bath at

$-2.5°$ C. and continue the manipulation of the stirrer until a super-cooling of the sample of 1.2° C. is observed. As a rule, by this time the liquid will begin to freeze, as may be noted by the rapid rise of the mercury thread. Manipulate the stirrer slowly and carefully three or four times as the mercury column approaches its highest point. By means of a suitable light weight cork mallet tap the upper end of the thermometer cautiously a number of times until the top of the mercury column remains stationary a couple of minutes. Taking the necessary precautions to avoid parallax, observe the exact reading on the thermometer scale and estimate to 0.001° C. When the observation has been satisfactorily completed make a duplicate determination, then remove the thermometer and stirrer and empty the water from the freezing tube.

Rinse out the test tube with about 25 cc. of the sample of milk, previously cooled to 10° C. or lower, measure into the tube 35 cc. of the milk, or enough to fairly submerge the thermometer bulb, and insert the tube into the apparatus. Maintain the temperature of the cooling bath at 2.5° C. below the probable freezing point of the sample. Make the determination on the milk, following the same technique as that employed in determining the freezing point of water. As a rule, however, it is necessary to start the freezing action in the sample of milk by inserting the freezing starter, carrying a fragment of ice, at the time when the mercury column has receded to 1.2° below the probable freezing point. A rapid rise of the mercury column results almost immediately. Manipulate the stirring device slowly and carefully two or three times while the mercury column approaches its highest point. Complete the adjustment of the mercury column in the same manner as in the preceding determination; then, avoiding parallax, observe the exact reading on the thermometer scale and estimate to 0.001° C. The algebraic difference between the reading obtained on the sample of water and the reading obtained on the sample of milk represents the freezing point depression of the milk.

The percentage of added water may be calculated as follows:

$$W = \frac{100\ (t - t^1)}{t}$$

in which,

t = the freezing point of normal milk which averages $-0.55°$ C.

t^1 = the observed freezing point of the sample.

W = added water, per cent by weight.

A more accurate formula which corrects for the fact that the added water should be calculated by using weights of solvent and not of solution, that is, one agreeing with usual custom and hence referring always to 100 g. of water, is:

$$W = \frac{t - t^1}{t} (100 - \text{T.S.})$$

in which,

T.S. = the percentage of total solids in the milk and the other symbols have the defined meaning.[18]

It is essential that the freezing point determination be made only on fairly fresh milk, owing to the fact that the development of acidity to the extent of 0.10 per cent beyond normal for fresh milk, approximately, 0.15 per cent, lowers the freezing point about 0.025° to 0.030° C. Therefore, milk that shows an acidity greater than 0.18 per cent as determined by the method detailed in the chapter on milk and cream, Chapter VI, should not be subjected to the test.

The percentage of added water in cream may be estimated in a similar manner. However, the following formula is used for the calculation:

$$W = \frac{t - t^1}{t} \%\ \text{serum in cream}$$

% serum in cream = 100% − (% fat + % protein) in which, if per cent protein is not estimated, it may be assumed to be 38 per cent of the solids-not-fat. The other symbols have the defined meanings.

REFERENCES

Taylor, "Treatise on Physical Chemistry," Van Nostrand (1931).
Findlay, "Practical Methods of Physical Chemistry," Longmans (1933).
Hawk and Bergeim, "Physiological Chemistry," Blakiston (1927).
Mahin, "Quantitative Analysis," McGraw-Hill (1924).
Fryer and Weston, "Oils, Fats and Waxes," Cambridge (1920).
Reilly, Rae and Wheeler, "Physico-Chemical Methods," Van Nostrand (1925).
Landolt-Long, "The Optical Rotation of Organic Compounds," Chemical (1902).
Radley and Grant, "Fluorescence Analysis," Van Nostrand (1933).

[18] Elsdon and Stubbs, *Analyst* 61, 382 (1936).

Leeds and Northrup, Bulletin No. 755 (1930).
Leeds and Northrup, Note Book No. 3 (1931).
Leeds and Northrup, Catalog No. EN.-96 (1937).
Perley, "Measurement and Control of Hydrogen Ion Concentration in Industrial Plants," Leeds and Northrup (1936).
Central Scientific Co., Bulletin No. 104 (1937).
Clark, "Determination of Hydrogen Ion," Williams and Wilkins (1920).
Bigelow, National Canners Association, Bulletin No. 27 L (1934).

CHAPTER III

COLORING MATTERS IN FOODS

The addition of coloring matters to foods has two main aims, one is to increase the attractiveness of the food and the other is to conceal damage or inferiority. That this is so is clear from the fact that there is additional cost in not only the dye, itself, but also in the process of adding the dye. Since manufacturers compete with other manufacturers no unnecessary costs may be permitted. Where color is added for a fraudulent purpose, governmental agencies generally do not tolerate their use. Color in such materials as confections, where the color is added, primarily, in order to increase the attractiveness, cannot be deemed objectionable. On the other hand, artificially coloring tomato paste, or ca[illegible] range drink, or French ice cream or similar products where [illegible] should naturally be present is definitely objectionable.

[illegible]oloring matters may be placed into 3 classes and in the order [illegible] importance:

1) Coal tar dyes or artificial coloring matters consisting of
   - (a) permitted and certified water soluble acid dyes and oil soluble dyes
   - (b) non-permitted and non-certified water soluble acid dyes, water soluble basic dyes and oil soluble dyes
2) Natural or vegetable colors
3) Mineral colors

Purpose of Certification

As ordinarily manufactured for textile or other industrial purposes, artificial dyes often contain impurities, some of which are harmless, whereas others are toxic. These impurities may not detract from the value of the dyes for industrial use, but they would be highly objectionable in a substance designed for human consumption. Practically all artificial dyes at one stage or another of their manufacture are treated with sulfuric or nitric acid, both of which are frequently contaminated

with arsenic. The vessels in which the dyes are made may also contain arsenic. Unless special precautions are taken the dyes may, therefore, be seriously contaminated with arsenic compounds. If the manufacturing processes are carried on in vessels of lead or copper, appreciable quantities of these metals may be dissolved and contaminate the dye. Harmful intermediates and organic compounds are present in most industrial dyes. Uncombined intermediates and other organic compounds produced by side reactions during manufacture, even though comparatively harmless, are objectionable in food dyes, except in very small proportions. The standards of purity set for certified dyes necessitate special precautions in their manufacture and purification, in order that appreciable quantities of objectionable substances may not be present in the finished dyes. Certification by the Food and Drug Administration, U. S. Department of Agriculture, implies not only that the dye itself is harmless, but that it is uncontaminated by poisonous substances.

The procedure of certification has been devised for the purpose of affording manufacturers of food colors and manufacturers of food products, as well as other consumers of food colors, a means of determining the suitability of these products for food use in so far as their purity and harmlessness are concerned. The use of color of any kind to conceal damage or inferiority in a food product is defined by the United States Federal Food and Drugs act as an adulteration and, when damage or inferiority is concealed, the employment of artificial color is not permissible, even though certified colors are used and their presence is declared on the label. In general, where colors are legitimately used in foods and beverages a statement on the label of the presence of artificial color is required except, as for example, in candies.

Permitted Dyes

The following coal tar colors are known as permitted dyes. If analysis by the Food and Drug Administration, U. S. Department of Agriculture shows that they comply with the standards of purity set by that bureau, they are then termed certified dyes.

Red Shades:

80 Ponceau 3R
Ponceau SX
184 Amaranth
773 Erythrosine

Orange Shades:

150 Orange I
Sunset Yellow FCF

Yellow Shades:

10 Naphthol Yellow S
640 Tartrazine
22 Yellow AB
61 Yellow OB

Green Shades:

666 Guinea Green B
670 Light Green SF Yellowish
Fast Green FCF

Blue Shades:

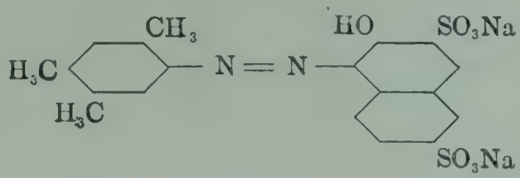
1180 Indigotine
Brilliant Blue FCF

Yellow AB and Yellow OB are known as oil soluble dyes, because they are soluble in oils but insoluble in water. The other 13 colors are soluble in water. The original list[1] of seven permitted dyes, namely, Amaranth, Ponceau 3R, Erythrosine, Orange I, Naphthol Yellow S, Light Green SF Yellowish and Indigotine was selected after a critical study of the reports of pharmacological tests on the more important dyestuffs. The other eight dyes were added to the list after appropriate pharmacological and toxicological tests had proved them to be harmless. The number opposite each color is that of the dye as listed in the Colour Index of 1924, published by the Society of Dyers and Colourists of England. Where no number is given the dye is one which has been especially developed for use as a food color and has not yet been included in the Colour Index.

[1] Hesse, U. S. Dept. Agr., Bur. Chem., Bull. No. 147 (1912).

Composition, Preparation and Nomenclature of the Certified Dyes [2,3]

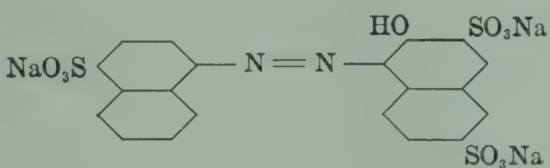
Ponceau 3R (80), a monazo dye, is the sodium salt of ψ cumene-azo-β-naphthol 3, 6 disulfonic acid. It is prepared by coupling diazotized cumidine with β naphthol 3, 6 disulfonic acid.



$C_{19}H_{16}N_2O_7S_2Na_2$

PONCEAU 3R

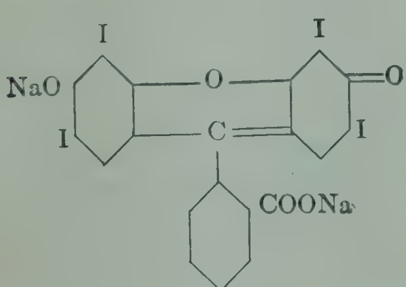
Amaranth (184), a monazo dye, is the sodium salt of 4-sulfo-α-naphthalene-azo-β-naphthol 3, 6 disulfonic acid. It is prepared by coupling diazotized α naphthylamine-4-sulfonic acid with β naphthol 3, 6 disulfonic acid.



$C_{20}H_{11}N_2O_{10}S_3Na_3$

AMARANTH

Erythrosine (773), a xanthene dye, is the sodium or potassium salt of tetraiodo-fluorescein, or of hydroxytetraiodo-o-carboxyphenylfluorone. It is prepared by the iodation of fluorescein in aqueous or alcoholic solution.



$C_{20}H_6O_5I_4Na_2 + H_2O$

ERYTHROSINE

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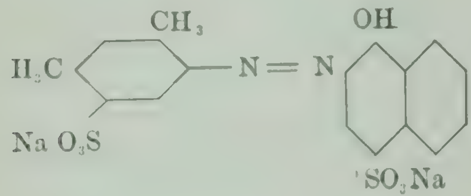
2 Ambler, Clarke, Evenson and Wales, U. S. Dept., Agr. Bull. No. 1390 (1927).

3 Evenson and Herrick, U. S. Dept. Agr., Bull. No. 1390 supplement No. 1 (1930).

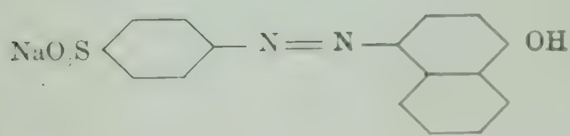
Ponceau SX, a monazo dye, is the disodium salt of the product obtained by coupling diazotized 1-amino-2, 4-dimethylbenzene 5-sulfonic acid with 1-naphthol-4-sulfonic acid.



$C_{18}H_{14}N_2O_7S_2Na_2$

PONCEAU SX

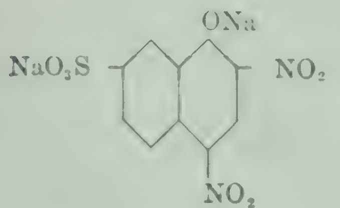
Orange I (150), a monazo dye, is the sodium salt of p-sulfobenzeneazo α naphthol. It is prepared by coupling diazotized sulfanilic acid with α naphthol.



$C_{16}H_{11}N_2O_4SNa$

ORANGE I

Naphthol Yellow S (10), a nitro dye, is the sodium salt of 2, 4 dinitro-α-naphthol-7-sulfonic acid. It is prepared by the action of nitric acid on α naphthol-2, 4, 7-trisulfonic acid, α naphthol-4, 7-disulfonic acid or α naphthol-2, 7-disulfonic acid or more usually the nitroso compound of the latter.

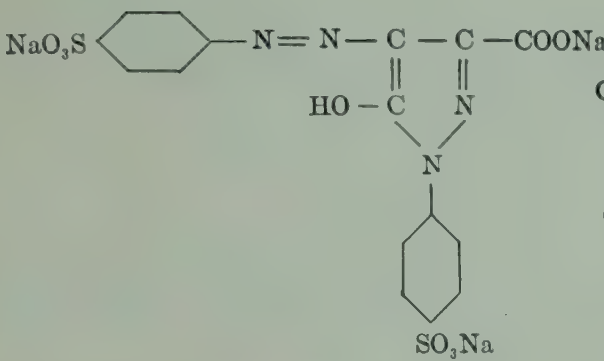


$C_{10}H_4N_2O_8SNa_2 + 3H_2O$

NAPHTHOL YELLOW S

Tartrazine (640), a pyrazolone dye, is the sodium salt of 4-p-sulfobenzeneazo-1-p-sulfophenyl-5-hydroxypyrazol-3-carboxylic acid. It may be prepared by a number of methods one of which is to couple diazotized sulfanilic acid with oxalaceticether, condense the product with phenyl-

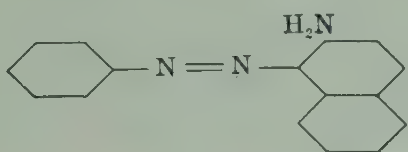
hydrazine-p-sulfonic acid and then hydrolyze the ester with sodium hydroxide solution.



$C_{16}H_9N_4O_9S_2Na_3$

TARTRAZINE

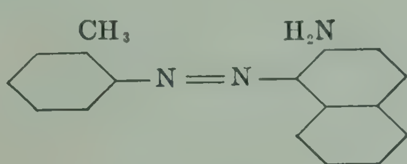
Yellow AB (22), an oil soluble monazo dye, is benzeneazo-β-naphthylamine. It is prepared by coupling diazobenzenechloride with β naphthylamine.



$C_{16}H_{13}N_3$

YELLOW AB

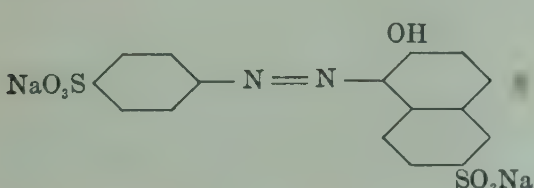
Yellow OB (61), an oil soluble monazo dye, is o-tolueneazo-β-naphthylamine. It is prepared by coupling diazotized o-toluidine with β naphthylamine.



$C_{17}H_{15}N_3$

YELLOW OB

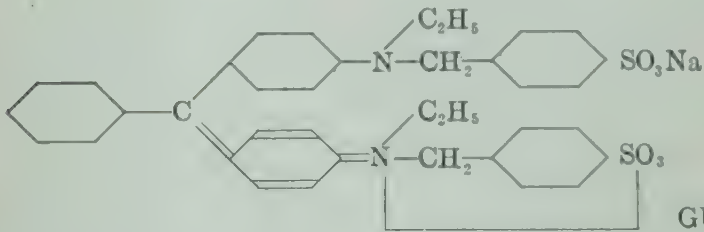
Sunset yellow FCF, a monazo dye, is the disodium salt of the product obtained by coupling diazotized sulfanilic acid with β naphthol-6-monosulfonic acid.



$C_{16}H_{10}N_2O_7S_2Na_2$

SUNSET YELLOW FCF

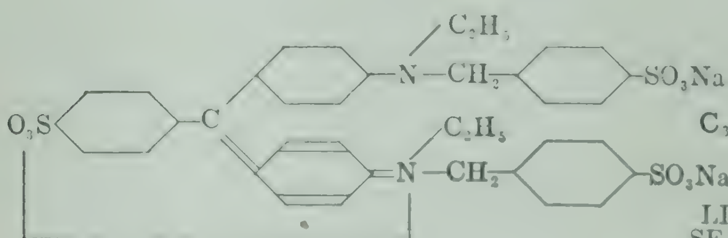
Guinea Green B (666), a triphenylmethane dye, is the sodium salt of dibenzyldiethyldiaminotriphenylcarbinoldisulfonic acid anhydride or of p-sulfobenzylethylaminofuchsonbenzylethylimonium sulfonate. It is prepared as follows: Condense benzaldehyde with benzylethylaniline sulfonic acid; oxidize the dibenzyldiethyldiaminotriphenylmethane disulfonic acid formed and convert to the sodium salt.



$C_{37}H_{35}N_2O_6S_2Na$

GUINEA GREEN B

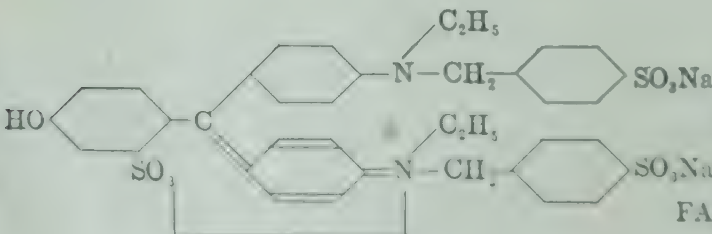
Light Green SF Yellowish (670), a triphenylmethane dye, is the sodium salt of dibenzyldiethyldiaminotriphenylcarbinoltrisulfonic acid anhydride, or the sodium salt of p-sulfobenzylethylamino-p'-sulfofuchsonbenzylethylimonium sulfonate. It is made by the condensation of benzylethylaniline with benzaldehyde, sulfonation of the product to the trisulfonic acid and the conversion of the resulting compound into the mono or disodium salt.



$C_{37}H_{34}N_2O_9S_3Na_2$

LIGHT GREEN
SF YELLOWISH

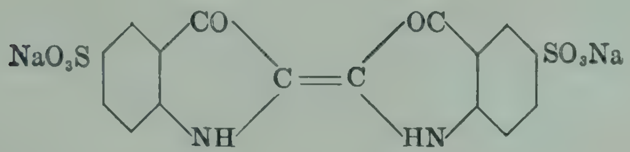
Fast Green FCF is a triphenylmethane dye and differs from guinea green B in that p-hydroxy-o-sulfobenzaldehyde is substituted for benzaldehyde.



$C_{37}H_{34}N_2O_{10}S_3Na_2$

FAST GREEN FCF

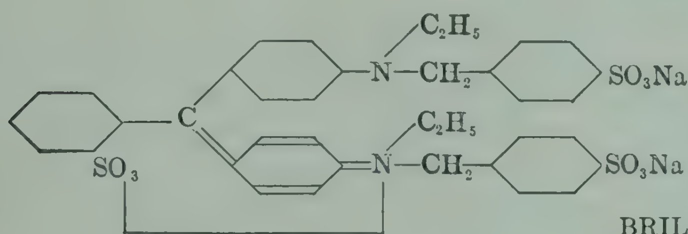
Indigotine (1180) or sodium indigodisulfonate or indigo carmine is the sodium salt of the disulfonic acid of indigo. It is prepared by sulfonating indigo.



$C_{16}H_8N_2O_8S_2Na_2$

INDIGOTINE

Brilliant Blue FCF is a triphenylmethane dye and is the disodium salt of the product made by condensing benzaldehyde-o-sulfonic acid with ethylbenzylaniline sulfonic acid.



$C_{37}H_{34}N_2O_9S_3Na_2$

BRILLANT BLUE FCF

Short Method for Water Soluble Acid Dyes

There are hundreds of dyes listed in Schultz, Julius and Green and in the Colour Index but the food analyst actually need look only among a very small number. The reason is that certified dyes are comparatively inexpensive because of increase in volume of manufacture. Furthermore, a small amount of artificial dye goes a long way. Consequently, only seldom does the analyst meet non-permitted colors. In fact the analyst has little means of concluding whether a certified or non-certified but permitted color has been used in a food product except by analyzing the dry color which in most cases can be obtained from the manufacturer of the food product. The dry color may then be carefully analyzed for purity. The use of coal tar colors follows the price scale very closely. For example, as the price of eggs goes up, so does the tendency for use of yellow shades of coal tar dyes increase.

Water soluble acid dyes have the property of dyeing wool in acid solutions. The dye may then be stripped from the wool by boiling in ammoniacal solution and subsequently may be redyed on wool in acid solution. A short general method for detecting water soluble acid coal

tar colors based on these properties is the following. Comminute 20–100 grams of the foodstuff according to the amount of color and mix with about 300–500 cc. of water, or if the material is soluble, dissolve the same proportion, if possible, in 300–500 cc. of hot water. Make slightly alkaline with ammonia, allow to stand for one or two hours so that the dye may be stripped from the foodstuff. Filter or decant through cheese-cloth or through a wire gauze made into a cone to fit a funnel. Make the filtrate slightly acid with hydrochloric acid, adding sufficient water to adjust the volume to about 500 cc. Add a 6–8 inch length of white wool yarn and bring the solution to a boil. Boil cautiously from 10 to 30 minutes. Remove the wool and wash thoroughly with cold water. Place in a beaker, add 25 cc. of water, 5 drops of strong ammonium hydroxide and boil to strip the color. Remove the wool, dilute the solution to 350 cc. and make acid by adding 10 drops of hydrochloric acid. Add a fresh 6 inch strip of white wool yarn and boil cautiously for 10–30 minutes. Remove the wool, wash thoroughly with cold water and then press dry. If the wool is colored, the food product probably contains a coal tar dye. Perform the spot tests with concentrated hydrochloric acid, concentrated sulfuric acid, 10 per cent sodium hydroxide solution, and 12 per cent ammonium hydroxide solution. Compare the reactions with Table 3 and identify the dye. If the spot tests are inconclusive, the wool has probably been dyed with a mixture of colors and one must proceed with the method of mixtures detailed below. It is possible at times that vegetable colors such as archil or turmeric may dye, strip and redye, and cognizance of such possibility must be taken before drawing conclusions. If orange I is suspected, in this short method, do not dilute the solution containing the stripped color of the first piece of wool. Make the 25 cc. barely acid with hydrochloric acid and dye on wool by warming alone, otherwise the orange I may be destroyed.

If a mixture of dyes is suspected, it is convenient to dye eight to ten strips of wool directly, then strip the pieces with ammonia water in a small volume and proceed as detailed in the "scheme for separation of permitted coal tar water soluble dyes."

Systematic Examination of Water Soluble Dyes

If the short method is inconclusive and there is reason to suspect that the coloring matter is not natural to the foodstuff a more comprehensive treatment is necessary. These methods make use of the law of partition

which states that a solute will distribute itself between a mixture of immiscible solvents at constant temperature in a constant ratio dependent on the respective solubility of the solute in each solvent. By the addition of salt or acid the dyes are made more or less soluble in the aqueous layer and correspondingly less or more soluble in the other solvent. By the addition of gasoline or carbon tetrachloride, the dyes are made less soluble in the immiscible solvent and more soluble in water.

Prepare a water solution of the coloring matter and strain as directed above. If the material is not soluble, comminute or grind and to 20–100 g. add 100 cc. of hot water, make slightly ammoniacal, allow to stand in a warm place for 2 hours and decant through a wire skimmer or sieve. Solid materials may be digested with 70 per cent alcohol containing 1 to 5 per cent ammonium hydroxide solution, although there is little advantage over steeping in ammoniacal water solution except to obtain traces of color. The alcohol, whether added for extractive purposes or whether present as an alcoholic sample being examined for color, must be removed by evaporation on a steam bath.

The details for the systematic examination of water soluble dyes by separation by immiscible solvents are abstracted from the methods of the Association of Official Agricultural Chemists, 4th edition (1935). The numbers in parentheses and brackets following the name of a dye represent in the first instance the number of that dye as listed in "A Systematic Survey of the Organic Colouring Matters" founded on the German edition of Drs. G. Schultz and P. Julius, 1904, by Arthur G. Green, while the second number designates the number as listed in the Society of Dyers and Colourists' "Colour Index" 1st edition, January, 1924.

In the following methods, where multiple extractions with a supernatant solvent are to be made, Jacobs-Singer separatory extraction flasks are most convenient for this purpose.

Basic Dyes—Most basic dyes may be separated from mixtures by making alkaline with 10 per cent sodium hydroxide solution and shaking with ether. Use the sample prepared as directed above. Separate the ether layer which may or may not be colored; wash it twice with a few cc. of water to remove excess of alkali; and shake with acetic acid (1 : 18), which will take up any dye present and form a colored solution. Although this treatment may, to some extent, alter the common basic colors, it can be used for the detection of methyl violet B (451) [680], magenta (448)

[677], bismark brown (197) [331], malachite green (427) [657], and rhodamine B (504) [749]. With care auramine (425) [655] also may be separated in this way, though it is quickly decomposed on standing in alkaline solution.

Acid Dyes—The following short procedure is often convenient for the examination of mixtures of acid dyes: Make the prepared sample strongly acid by adding ½ its volume of hydrochloric acid and shake 3 times with portions of amyl alcohol. Separate and combine the amyl alcohol solutions and wash by shaking with successive portions of ½ its volume of water, reserving the portions in separate bottles. Because of the varying acid content of the amyl alcohol these washings will show a regular decrease in acidity, and the coloring matters will appear in maximum quantity in the different fractions according to their respective solubilities. Ponceau 6R (108) [186] is washed out chiefly while the acidity is still high, approximately normal. Amaranth (107) [184], brilliant scarlet (106) [185], tartrazine (94) [640], sunset yellow FCF, orange G (14) [27], and soluble blue (480) [707] appear when the washings have an acidity of about 0.25 *N*, and palatine scarlet (53) [77], ponceau 2R (55) [79] and 3R (56) [80], ponceau SX, naphthol yellow S (4) [10], cochineal (706) [1239], crystal ponceau (64) [89], and azorubine A (103) [179] between 1/16 *N* and 1/256 *N*. When practically all the acid is removed, orange I (85) [150], orange II (86) [151], and croceine orange (13) [26] begin to wash out, and less readily, orange IV (88) [143] and metanil yellow(95) [138]. Finally the unsulfonated coloring matters, such as erythrosine G (516) [772], erythrosine B (517) [773], and the rose bengals (520) [777] and (523) [779] are removed very slowly by water or not at all unless the solvent is diluted with gasoline and the dyes are removed with water containing a few drops of ammonium hydroxide solution. Acid yellow (8) [16] and brilliant yellow S (89) [144] are not very uniform in composition. They are partially taken up by amyl alcohol from acid solution and appear chiefly in the first washings. Indigotine (692) [1180] behaves somewhat similarly.

Transfer the colored washings to beakers and dilute with a large amount of water. Add a strip of wool and heat to boiling. Boil for a few minutes and then remove the wood, wash thoroughly with cold water and perform the spot tests to verify the identification of the dye.

SCHEME FOR SEPARATION OF PERMITTED COAL TAR WATER SOLUBLE DYES

<table>
<tr><td colspan="5">Original color solution made up to 10% salt solution with NaCl and containing 1 volume of HAc to 7 volumes of solution. Extract in 50 cc. volumes with 3—50 cc. portions of amyl alcohol.</td></tr>
<tr><td colspan="2">Amyl alcohol layer. Wash with successive portions of 25 cc. of 5% NaCl solution until colorless and return washings to original solution. This layer now may contain orange I, guinea green B, erythrosine. Add an equal volume of gasoline and wash with 25 cc. portions of water.</td><td colspan="3">Aqueous layer and washings. Acidify with HCl, one volume of acid to 40 volumes of solution and extract in 50 cc. portions with 3—50 cc. portions of amyl alcohol.</td></tr>
<tr><td rowspan="2">Aqueous extract contains orange I and guinea green B.</td><td rowspan="2">Shake amyl alcohol-gasoline layer with 0.1 N NaOH or NH_4OH (1 : 9). Aqueous extract contains erythrosine.</td><td rowspan="2">Amyl alcohol layer. Wash with 25 cc. portions of 0.25 N HCl and return washings to original solution. Wash amyl alcohol layer with 25 cc. portions of water. Aqueous layer may now contain ponceau 3R, ponceau SX, naphthol yellow S.</td><td colspan="2">Aqueous layer and washings. Extract in 50 cc. volumes with 3—50 cc. portions of alpha dichlorhydrin.</td></tr>
<tr><td>Alpha dichlorhydrin layer. Wash with 20 cc. portions of 25% NaCl solution and return washings to original solution. Dilute with 2 volumes of CCl_4 and wash with water until all color is extracted. Aqueous layer may now contain light green SF yellowish, fast green FCF and brilliant blue FCF.</td><td>Aqueous layer and washings. Acidify further with HCl, 1 volume acid to 40 volumes of solution. Extract in 50 cc. volumes with 3—50 cc. portions of amyl alcohol. Discard aqueous layer. Wash out dyes from amyl alcohol layer with 25 cc. portions of water. Aqueous layer may now contain the remaining coloring matters, indigotine, amaranth, tartrazine and sunset yellow FCF.</td></tr>
</table>

For separation of remaining groups refer to the separations detailed in the text.

Separation of the Permitted Coal Tar Water Soluble Dyes

The following directions and those given in the scheme are general and the proportions of amyl alcohol, dichlorhydrin and other reagents used should be varied with the amount of color present in the aqueous solution. To the solution containing the color add sufficient 25 per cent salt solution to make the concentration about 10 per cent and 1 part of acetic acid to every 7 parts of solution. Extract with 3–50 cc. portions of amyl alcohol. Draw off the lower layer and reserve for further treatment. Wash the amyl alcohol extract in rotation with 25 cc. portions of 5 per cent salt solution until the washings are colorless or nearly so. Add the washings to the original aqueous solution. Dilute the amyl alcohol extract with an equal volume of gasoline or petroleum ether and wash with 25 cc. portions of water until all color is extracted. The coloring matters obtained are orange I and guinea green B. For their separation see page 89. Treat the amyl alcohol gasoline solution with 10 cc. portions of 0.1 *N* sodium hydroxide solution or with 10 cc. portions of ammonium hydroxide solution (1 : 9), which will remove erythrosine. The original solution and washings, from which the 3 named dyes were removed, are acidified with hydrochloric acid (1 volume acid to 40 volumes of solution) and extracted in 50 cc. volumes with three 50 cc. portions of amyl alcohol. Reserve the lower aqueous layer for further treatment. Wash the amyl alcohol extract with 25 cc. portions of 0.25 *N* hydrochloric acid until the washings are colorless or nearly so. Combine the washings with the aqueous solution above. The amyl alcohol is extracted with several 25 cc. portions of water until all color is extracted. The coloring matters obtained are ponceau 3R, ponceau SX, and naphthol yellow S. For their separation see page 89. The original solution and washings, from which the 6 named dyes were removed, are treated in 50 cc. volumes with three 50 cc. portions of α dichlorhydrin. Reserve the upper aqueous layer for further treatment. Wash the dichlorhydrin extract in rotation with several 20 cc. portions of 25 per cent salt solution. Combine the washings with the aqueous solution above. Dilute the dichlorhydrin extract with 2 volumes of carbon tetrachloride and extract with several 25 cc. portions of water until all color is extracted. The coloring matters obtained are light green SF yellowish, fast green FCF and brilliant blue FCF. For their separation see page 89. Further acidify the original solution and washings, from which the 9 named dyes were removed, with hydrochloric acid (1 volume of acid to 40 volumes of solution), and

extract in 50 cc. volumes with three 50 cc. portions of amyl alcohol. If the color intensity of the solution was not too strong, all coloring matter should have been extracted by the solvent. Discard the lower colorless or nearly colorless layer and wash out the dyes from the amyl alcohol extract in rotation with several 25 cc. portions of water until all color is extracted. The coloring matters obtained are indigotine, amaranth, tartrazine, and sunset yellow FCF. For their separation see page 90.

Orange I and Guinea Green B. Extract the combined colors with two 20 cc. portions of dichlorhydrin. Discard the colorless upper aqueous layer, dilute the solvent with 2 volumes of carbon tetrachloride, and extract out orange I in rotation with several 10 cc. portions of water, and guinea green B with several 10 cc. portions of 25 per cent alcohol.

Ponceau 3R, Ponceau SX, and Naphthol Yellow S. Acidify the combined colors with hydrochloric acid (1 part acid to 10 parts of solution), and extract the naphthol yellow S with two 20 cc. portions of washed ethyl acetate or amyl acetate. Ponceau 3R and ponceau SX are not extracted appreciably and remain in the aqueous layer. Wash the solvent with 5 cc. portions of N hydrochloric acid to remove traces of the ponceaus. Naphthol yellow S is removed from the combined ethyl acetate or amyl acetate with 5 cc. portions of ammonium hydroxide solution (1:9). Extract the remaining ponceau solution with 20 cc. portions of amyl alcohol and wash out excess of acid twice with a few cc. portions of water. Dilute the amyl alcohol with an equal volume of gasoline, or petroleum ether, and remove the color with small volumes of water. Treat 10 cc. of this solution with 1 cc. of hydrochloric acid, 2 cc. of strong bromine water and lastly 3 cc. of saturated hydrazine sulfate solution and immediately pour into a test tube containing 10 cc. of 2 N sodium carbonate solution and 2 drops of 1 per cent alcoholic α naphthol. A light orange solution indicates ponceau 3R. A deep brownish red solution indicates ponceau SX. Add to the solution 5 cc. of ether, mix well and draw off the lower aqueous layer which, if colored, contains ponceau SX. To the ethereal extract add an equal volume of hydrochloric acid. The formation of a purplish solution confirms the presence of ponceau 3R.

Light Green SF Yellowish, Fast Green FCF and Brilliant Blue FCF.

Treat the combined colors with an equal volume of 2 *N* sodium carbonate solution and extract in 25 cc. volumes with two 50 cc. portions of *N* butyl alcohol. Draw off the lower aqueous layer containing the fast green FCF and wash out the last traces from the solvent with 25 cc. portions of 2 *N* sodium carbonate solution. Reserve the washings and add to the aqueous solution for confirmatory tests. Light green SF yellowish is colorless in the solvent while brilliant blue FCF imparts a bluish green to it. To prove the presence of light green SF yellowish in the presence of brilliant blue FCF proceed as follows: Dilute the solvent with an equal volume of gasoline and remove color with small portions of water. Treat 20 cc. of solution with 4 cc. of 10 per cent sodium hydroxide solution and boil for 5 minutes. Brilliant blue FCF is changed to a red phase, while light green SF yellowish is changed to a yellow. Acidify with 10 cc. of glacial acetic acid, which changes brilliant blue FCF to a violet and light green SF yellowish to a green. Treat with about 3 g. of zinc dust and heat until the solution is decolorized. Filter, make slightly alkaline with ammonium hydroxide and later make acid with acetic acid. Bring to a boil. In the presence of light green SF yellowish a deep green solution is formed while brilliant blue FCF remains colorless.

Indigotine, Amaranth, Tartrazine and Sunset Yellow FCF. To separate the indigotine, heat a small portion of the solution, which should be neutral or faintly acid, to boiling and add a few crystals of sodium hyposulfite, $Na_2S_2O_4$, until all the dyes are reduced. On adding a few drops of glacial acetic acid and shaking with air the indigotine is quickly restored, while amaranth, tartrazine, and sunset yellow FCF are destroyed. If a positive test for indigotine is obtained, add to the remainder of the mixed dye solution several decigrams of urea, heat, and while the mixture is boiling add 1 or 2 drops of 10 per cent sodium nitrite solution. Indigotine is converted to the pale yellow isatine sulfonate, while amaranth, tartrazine, and sunset yellow FCF are but little affected. Acidify the resultant mixture with sulfuric acid (1 : 4), using 1 part of the dilute acid to 10 parts of solution. Extract in 25 cc. portions with three 50 cc. portions of *N* butyl alcohol. Draw off the lower layer and pass successively through all the funnels. Reserve the aqueous layer if colored, if not colored, discard. Prepare the following solution: 13.5 cc. of sulfuric acid, 100 g. of anhydrous sodium sulfate, and sufficient water to make 1 liter. Extract the *N* butyl alcohol successively with

25 cc. portions of the solution until washings are colorless. Reserve them for amaranth and tartrazine. Dilute the *N* butyl alcohol with an equal volume of gasoline and remove sunset yellow FCF with water. Confirm with dyeing tests and wet reactions.

Acidify the reserved solution with hydrochloric acid (1 volume of acid to 20 of solution) and extract with two 30 cc. portions of amyl alcohol. This will extract both amaranth and tartrazine while the isatine compound, being less readily extracted, remains in the lower layer and is discarded. Remove the coloring matter with several 10 cc. portions of water. To a portion of the solution add 5 drops of ammonium hydroxide solution and a few crystals of sodium hyposulfite, $Na_2S_2O_4$. This treatment will destroy amaranth completely, leaving tartrazine practically unaltered. Add an excess of hydrochloric acid and speedly extract the dye with a small amount of amyl alcohol, from which solution tartrazine can be removed with 0.25 *N* hydrochloric acid. Treat another 10 cc. portion of the neutral dye solution in a test tube with 2 cc. of 20 per cent ammonium chloride and 1 cc. of 25 per cent potassium cyanide solution and heat in a boiling water bath for 5 minutes. Cool rapidly and acidify with 2 cc. of hydrochloric acid and extract with 10 cc. of amyl alcohol. [Caution]. Draw off the lower layer and discard. Remove the tartrazine with 5 cc. portions of 0.25 *N* hydrochloric acid, amaranth is converted to a lower sulfonated dye, and is not removed at that acid concentration. Dilute the solvent with an equal volume of gasoline or petroleum ether and extract the dye with small volumes of water. Amaranth is modified to a brownish red dye.

Identification of Dyes by Spot Tests

Transfer the separated coloring matter to wool, or silk in the case of oil soluble dyes by boiling as directed on page 84. Cut off four small pieces from the strip of dyed wool yarn or silk thread and place in the depressions of a white porcelain spot plate. Moisten the pieces with concentrated hydrochloric acid, concentrated sulfuric acid, 10 per cent sodium hydroxide solution, and 12 per cent ammonium hydroxide solution, respectively. At the same time, repeat with four pieces of known fiber of similar concentration. Compare the reaction with those listed in Table 3.

TABLE 3. COLOR CHANGES PRODUCED ON DYED FIBERS BY VARIOUS REAGENTS[4]

Coloring Matter	Conc. HCl	Conc. H_2SO	10% NaOH	12% NH_4OH
Rhodamine B	orange	yellow	bluer	bluer
Rose Bengal	almost decolorized	orange	no change	no change
Archil	red	reddish-brown	violet	violet
Magenta	yellowish-brown	yellowish-brown	decolorized	paler
Acid Magenta	almost decolorized	yellow	decolorized	decolorized
Palatine Red	darker	blue	dull brown	little change
Bordeaux B	violet	blue	brick red	little change
Amaranth	slightly darker	violet to brownish	dull brownish to orange red	little change
Azorubine A	little change	violet	red	red
Erythrosine	orange yellow	orange-yellow	no change	no change
Ponceau 6RB	blue	blue	dull violet-red	little change
Ponceau 6R	violet red	violet	brown	orange-red
Crystal Ponceau	red	violet	rull brown	little change
Ponceau 3R	little change	little change	dull orange	little change
Ponceau SX	deeper red	deeper red	orange-yellow	orange-yellow
Sudan III†	violet, then brown	green	violet-red	little change
Safranine	greenish blue	green	red	red
Brilliant Scarlet	red	violet red	yellowish brown	orange-red
Ponceau 2R	little change	little change	brownish yellow	no change
Palatine Scarlet	darker	violet red	brownish yellow	no change
Erythrosine G	yellow-orange	yellow-orange	no change	no change
Sudan II†	red	violet red	little change	no change
Sudan I†	orange-red	red	redder	no change
Cochineal	little change	little change	violet red	violet red
Bismarck Brown	redder, darker	browner	yellower	yellower
Bismarck Brown R	redder, darker	browner	yellower	yellower
Orange I	violet	violet	red, dark	red, dark
Orange II	red	red	dull red	no change
Crocein Orange	orange red	orange	slightly darker	no change
Orange G	little change	orange	dull brownish-red	no change

† Oil-soluble.

[4] Mathewson, U. S. Dept. Agr., Bull. No. 448 (1917).

TABLE 3—*Continued*

Coloring Matter	Conc. HCl	Conc. H_2SO	10% NaOH	12% NH_4OH
Yellow OB†	red	violet	little change	no change
Yellow AB†	red	violet	little change	no change
Sudan G†	orange-yellow	brownish yellow	orange-yellow	no change
Butter Yellow†	violet-red	orange-yellow	no change	no change
Aniline Yellow†	violet-red	orange-yellow	little change	no change
Aminoazoorthotoluene†	dull orange	orange-yellow	little change	no change
Fluoresceine	little change	little change	green fluorescent	green fluorescent
Metanil Yellow	violet red	violet	no change	no change
Azoflavine	violet red	violet red	dull brown	little change
Acid Yellow	red	orange	little change	no change
Brilliant Yellow S	violet red	violet red	little change	little change
Tartrazine	slightly darker	slightly darker	little change	little change
Sunset Yellow FCF	redder	redder	browner	no change
Naphthol Yellow S	almost decolorized	very pale, dull brown	no change	no change
Auramine	decolorized	almost decolorized	decolorized	paler
Turmeric	red	reddish-brown	orange	orange
Quinoline Yellow	slightly darker	brownish-yellow	slightly paler	little change
Naphthol Green B	yellowish	brownish-yellow	no change	no change
Guinea Green B	pale orange-yellow	yellow-brown	decolorized	decolorized
Light Green SF yellowish	pale orange-yellow	yellow-brown	decolorized	decolorized
Fast Green FCF	orange	green to brown	blue	blue
Brilliant Blue FCF	yellow	yellow	no change	no change
Night Green 2B	pale orange-yellow	yellow-brown	decolorized	paler
Malachite Green	almost decolorized	almost decolorized	decolorized	decolorized
Erioglaucine A	yellow	pale, dull yellow or brown	slightly darker	little change
Patent Blue A	pale orange-yellow	green to brown	little change	little change
Soluble Blue	paler	brown	pale red	almost decolorized
Indigotine	slightly darker	darker	greenish-yellow	greenish-blue
Formyl Violet	pale orange-yellow	pale, dull orange	decolorized	decolorized
Methyl Violet	yellowish	yellowish	decolorized	almost decolorized
Nigrosine, Soluble	dull bluish	dull greenish	brownish red, paler	pale reddish

† Oil-soluble.

Common Mixtures—It is, of course, obvious that not all 13 water soluble dyes are going to be present in a foodstuff and consequently, the foregoing method is merely to be used as a general scheme, the analyst, skipping those parts that unquestionably do not enter into the method for the moment. The following are some of the combinations that are more common.

Egg shade:	tartrazine	+ orange I	
Greens:	tartrazine	+ indigotine	(pistachio)
	tartrazine	+ brilliant blue FCF	(lemon)
	tartrazine	+ fast green FCF	(lemon)
	tartrazine	+ guinea green B	(lemon)
	tartrazine	+ light green SF yellowish	(lemon)
Orange:	tartrazine	+ orange I	
	tartrazine	+ ponceau 3R	
	tartrazine	+ sunset yellow FCF	
	tartrazine	+ ponceau 3R + amaranth	
Brown:	ponceau 3R	+ orange I	(chocolate and
	ponceau SX	+ orange I	frankfurters)
Violet:	amaranth	+ indigotine	(grape color
	amaranth	+ brilliant blue FCF	in jams,
	amaranth	+ fast green FCF	jelly, etc.)
	amaranth	+ ponceau 3R + orange I	

The use of orange I is declining because a muddy color or precipitate develops in the presence of iron. The use of a particular dye or any other combination of dyes is very often a matter of price. Thus, erythrosine is seldom used because it is comparatively expensive; amaranth is used instead.

A simple means for extracting water soluble coloring matters from a food product is to take up the dye on 5 to 10 six inch pieces of white wool yarn, stripping in a small amount of water with the aid of ammonia and then going on with the systematic method.

Short Method for Oil Soluble and Natural Coloring Matters

Grind such materials as alimentary pastes, comminute other materials and mix thoroughly foodstuffs similar to mayonnaise and salad dressing. Place about 50–75 g. of the material in a bottle or beaker, if pasty, and wet with 10–15 cc. of alcohol. This aids in the breaking of emulsions and in the extraction of color. Add 100 to 150 cc. of ethyl ether and allow to stand for a few hours or overnight. Filter through filter paper. In the case of pasty materials, pour the supernatant ether layer through

the filter. Wash the residue with ether, pouring the washings through the filter. Catch the filtrate in an evaporating dish and evaporate off the ether and alcohol. The residue in the dish is fat or oil and color. Take up in petroleum ether or gasoline. Place one portion in a test tube and shake with a few ccs. of 13 N sulfuric acid. Allow to stand and separate, or centrifuge. A pink or orange color in the acid layer shows the presence of a coal tar dye. This color may be due to yellow OB or AB or some other oil soluble coal tar color. The probability is that if coal tar color is present it will be due to yellow OB or AB. If such color is present, or if this short test is inconclusive, proceed with the methods detailed under the sections, "Separation of yellow AB and OB" and "Separation of oil soluble dyes."

Return the other portion to the evaporating dish and evaporate off the petroleum ether or gasoline. Take up the residue in ethyl ether and place a portion in a test tube. Add a few cc. of ten per cent sodium hydroxide solution and shake. Allow to stand or centrifuge. If the sodium hydroxide layer is colored, a natural coloring matter is present. This may be due to annato or turmeric. Draw off the sodium hydroxide layer, if colored, with the aid of a small separatory funnel and divide into two portions. Dilute one portion with an equal amount of water and allow a piece of absorbent cotton to steep in the solution for a few hours or overnight. If the cotton after washing gently in water remains colored a straw hue which is turned pink or purple by a drop of stannous chloride solution, annato is confirmed. To the other portion, add sufficient 4 per cent boric acid in strong hydrochloric acid to make the mixture acid. A red color confirms the presence of turmeric.

If traces of color are present, the sodium hydroxide layer will scarcely be colored. Therefore this rapid test is not completely applicable for traces of color. The change from straw to pink, undergone by annato, is probably due to the acid of the stannous chloride solution and not to the stannous chloride, itself, for hydrochloric acid, acetic acid and sulfuric acid, all give this color reaction, although stannous chloride is not present. Some authorities believe that the purple color produced is due to the stannous chloride.

Evaporate off the ether in the remaining portion and take up in chloroform. Place 0.1 cc. in a micro test tube and add 9 drops of trichloroacetic acid solution (9:1). An intense blue color indicates the presence of carotene.[5] To verify, add to another 0.1 cc. portion 3–4 drops of anti-

[5] Levine and Bein, *Proc. Soc. Exptl. Biol. Med.* 32, 335 (1934).

mony trichloride. Again a blue color develops in the presence of carotene. A red color at this point in either of these two tests indicates ergosterol or some similar sterol.

Separation of Yellow AB and Yellow OB

The following details are given by the A. O. A. C. for the separation of yellow AB and OB. Extract a gasoline solution of these dyes 3 times with ½ its volume of 13 *N* sulfuric acid. Shake each acid extract successively with 2 portions of equal volume of low-boiling gasoline or petroleum ether, using the same 2 portions for each acid portion. Extract each of the 2 latter gasoline portions with 20 cc. of 13 *N* sulfuric acid, using the same acid portion successively for both gasoline portions. Finally extract the second of these gasoline portions with another 20 cc. portion of 13 *N* sulfuric acid. The original gasoline solution has now been shaken with acid 3 times, the next gasoline portion 4 times, and the third 5 times. Combine the acid extracts, dilute with water, re-extract with low-boiling gasoline, and evaporate the solvent. Yellow AB will be found in a practically pure state. Combine the gasoline solutions, original and subsequent solutions left after the acid washings, wash with small portions of water to remove excess of acid, and evaporate the solvent. The yellow OB will remain as a residue. This method is not absolutely quantitative, but it is sufficiently accurate to make a separation of either of the dyes with comparatively little contamination from the other. The following color test may be applied to the separated dyes to confirm their identity: Shake 5 cc. of a neutral gasoline solution of the dye in a test tube with 5 cc. of a mixture of 1 part of 40 per cent formaldehyde solution and 4 parts of acetic anhydride. Both coloring matters are extracted by the acetic anhydride, yellow AB giving in a few seconds a red colored solution, and yellow OB, under the same conditions, giving an orange colored solution.

Separation of Oil Soluble Dyes

If non-permitted oil soluble coal tar dye is suspected, one may proceed according to the following method from the A. O. A. C. or as outlined in a text dealing exclusively with identification of dyes. Prepare an alcoholic solution of the dye by applying one of the following methods to the oil or fat obtained by extraction with ether or gasoline if the nature of the substance requires it:

(a) Shake the oil or melted fat with an equal volume of alcohol, 90 per cent by volume, and wash the alcoholic extract with several portions of gasoline to free the coloring matter from fats. The alcohol, after separation, will contain aniline yellow, butter yellow, aminoazotoluene, auramine, sudans, yellow OB, yellow AB, etc., if present.

(b) Saponify 20 to 200 g. of the oil or fat with 0.5 *N* alcoholic potassium hydroxide solution, remove most of the alcohol on the steam bath, and extract the soap with ether or gasoline. Remove the dyes from the solvent with 10 cc. portions of a mixture containing 1 part of hydrochloric acid and 5 parts of glacial acetic acid. Most of the common dyes are removed by this treatment, though the digestion with strong alkali may cause some decomposition and make the extraction rather troublesome.

(c) Dilute 20 to 200 g. of the oil or melted fat with 1–2 volumes of gasoline and shake out successively with 2–4 per cent potassium hydroxide or sodium hydroxide solution, hydrochloric acid (1:3), and phosphoric acid-sulfuric acid mixture, prepared by mixing 85 per cent phosphoric acid with about 10–20 per cent sulfuric acid, by volume. The dilute alkali extracts sudan G (10) [23] and annatto (709) [1241]. The dilute hydrochloric acid extracts aniline yellow (7) [15], aminoazotoluene (—) [17], and butter yellow (16) [19]. The first two form orange-red, the latter cherry-red solutions in this solvent. The phosphoric acid mixture is necessary for the extraction of sudan I (11) [24], sudan II (49) [73], sudan III (143) [248] and the homologue of the last, sudan IV (—) [258]. Benzeneazo-β-naphthylamine (—) [22] and homologues also come in this group, though they readily undergo chemical changes in the strongly acid mixtures. The procedure is not very suitable in the presence of auramine, but this dye is seldom found in oils. Neutralize the alkaline and the dilute hydrochloric acid solutions, dilute the phosphoric acid mixture and partially neutralize, cooling the liquid during this operation. Extract the dyes by shaking with ether or gasoline.

For the direct dyeing test use the alcoholic solution obtained as directed under (a). Evaporate to dryness the ether or gasoline solutions obtained as directed under (b) and (c) and dissolve the residue in 10–20 cc. of 95 per cent alcohol. To the alcoholic solution add some strands of white silk and a little water and evaporate on a steam bath until the alcohol has been removed or the dye is taken up by the silk. The dyeing test is sometimes unsatisfactory, and in all cases a small

portion of the alcoholic solution should be tested by treating with an equal volume of hydrochloric acid and stannous chloride solution. The common oil soluble coal tar dyes are rendered more red or blue by the acid and are decolorized by the reducing agent. Most of the natural coloring matters become slightly paler with the acid and are little changed by the stannous chloride solution.

The dyed strands of silk may then be identified by performing the spot tests previously outlined, checking by using known strands of about equal intensity and referring to the color reactions listed in Table 3.

Separation of Natural Coloring Matters

Natural coloring matters show little tendency to dye animal fibers. However, they may be extracted by various solvents, re-extracted with water and subjected to color tests with divers reagents on portions of the aqueous solution. From neutral solutions ether extracts carotin, xanthophyll, the pigments found in leaves, fats and oils, egg yolk, carrots, etc., the coloring matter of tomatoes and paprika, and green chlorophyll. The coloring matter remains in the ether solution on shaking with normal sodium hydroxide solution or normal hydrochloric acid, no apparent change taking place, although chemically the substances may be altered more or less by this treatment.

Annatto, turmeric, alkanet and the red dyewoods are extracted by ether from acid solution almost completely. The coloring matters of this group are easily removed from the ether by shaking with alkaline solutions.

Amyl alcohol extracts largely the coloring matters of logwood, archil, saffron, and cochineal, from slightly acid solutions. Amyl alcohol extracts in relatively small proportions caramel and the anthocyans constituting the red coloring matter of the most common fruits.

Mineral Colors

The use of mineral colors in foods is very small, primarily because many of them are poisonous. These mineral colors are called pigments. Lakes are organic substances combined with metals or metallic salts, and are considered with mineral colors because of their metallic content. Examples of pigments are lampblack or other forms of carbon, prussian blue, talc, ochres, umbers and ultramarine blue. These may all be

identified by the usual tests for metals and other elements as for example carbon in lampblack and sulfur in ultramarine blue. The analysis of these materials will be discussed in some detail in the section on metals in foods. It must be noted that certain natural coloring matters such as chlorophyll derivatives contain metallic elements as magnesium.

For special tests for the water soluble acid and basic dyes, the oil soluble coal tar colors and the natural and mineral coloring matters, the reader is referred to any standard text on the analysis and identification of dyes. Some of these will be found in the bibliography at the end of the chapter. In these texts the reader will also find complete methods for the analysis of the dry dye powder.

Detection of Color on Oranges

The use of ethylene gas to develop a riper color and to hasten ripening of oranges has been supplemented by another means of augmenting the desirability and saleability of oranges, namely, the use of color. Oranges that are off-color or spotty or have other slight imperfections, may be made to appear the equal of better oranges by judicious use of color.

The addition of color to the skin of oranges may easily be detected by a method based on the solution of the dye in chloroform, dyeing the color on silk from 70 per cent alcohol solution and subsequent identification of the color by means of spot tests.

Place the orange in a funnel. Wash with a stream of chloroform directed from a wash bottle, rotating the orange at intervals so that the entire orange is washed with the chloroform. Catch the washings in a 400 cc. beaker. Wash two or three more oranges in a similar manner and catch the washings in the same beaker. Evaporate the chloroform solution to dryness on a water bath. Dissolve the color in 70 per cent alcohol and dye the color on silk by steeping the silk in the alcohol solution of the color on a water bath. Replenish the alcohol from time to time by the addition of 70 per cent alcohol. Remove the silk and wash thoroughly with water. Dry and apply spot tests.

A more exhaustive method is to dry the peel, extract the oil and color with ether or gasoline and then proceed as directed in the section, "separation of oil soluble colors." The dyes used are, generally, of the Sudan type, some of which are under consideration as permitted food colors.

Carotene [6]

The estimation of the carotene content of forage and feed provides an index to the vitamin A value of the forage or feed. The following method which, with slight modification, can be applied to similar materials, or to alimentary paste, isolates the carotene and then estimates the carotene by comparison with artificial standards.

Digest 2 to 6 g., according to the carotene content, of the sample with 40 to 120 cc. of saturated, aldehyde-free, alcoholic potassium hydroxide for 30 minutes under a reflux condenser. Cool, add 50 cc. of ethyl ether, shake, allow to settle and transfer the ether-alcohol layer to a separatory funnel. Wash the residue with small portions of alcohol and ether until the washings are colorless, and combine the washings with the first extract. Remove the chlorophyllins and flavones from the ether-alcohol mixture by adding 100 cc. of water to the separatory funnel, shaking and allowing the mixture to separate. Transfer the water layer to another separatory funnel and wash twice gently with ether. Combine the ether layers again and then continue washing with water until free from alkali and chlorophyllins.

Evaporate the ether solution containing the carotene and xanthophyll on a steam or water bath to remove the solvent. Extract the residue with three 10 cc. portions of petroleum ether and transfer to a separatory funnel. Remove the xanthophyll from the petroleum ether solution by washing with 20 cc. portions of 85 per cent methyl alcohol and then 90 per cent methyl alcohol. Transfer the petroleum ether layer to a volumetric flask, make to volume and compare in a colorimeter against Sprague's [7] dye standard or against 0.1 per cent potassium dichromate solution.[8]

REFERENCES

Methods of A. O. A. C. (1935).

Service and Regulatory Announcements, Food and Drug Administration, No. 3, Rev. 1, U. S. Dept. Agr. (1931).

"Colour Index," Society of Dyers and Colourists (1924).

Mulliken, "Identification of Pure Organic Compounds," Wiley (1916).

Green, "Analysis of Dyestuffs," Lippincott (1916).

Allen's Commercial Organic Analysis, 5th ed., Vol. 5, Blakiston (1927).

Green, "Organic Coloring Matters," Macmillan (1908).

Mathewson, U. S. Dept. Agr. Bull. No. 448 (1917).

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[6] Peterson, Hughes and Freeman, *Ind. Eng. Chem., Anal. Ed.* 9, 71 (1937).

[7] *Science* 67, 167 (1928).

[8] Munsey, *J. Assoc. Official Agr. Chem.* 20, 459 (1937).

CHAPTER IV

PRESERVATIVES IN FOODS

PRESERVATIVES may be defined as chemical agents which serve to retard, hinder, or mask the changes in food. The use of preservatives in foodstuffs is a practice of long standing. Some substances used as preservatives may in themselves be harmless. Thus sugar, nitrates, brine, vinegar, smoke, and alcohol, for example, are used in the preservation of food materials and no serious objection to their use in proper amount can be offered. Such substances are not generally classed as preservatives, although they have a decided germicidal, bactericidal or antifermentative action. However, if these substances are added to conceal damage or inferiority, strict interpretation of food laws would imply that even such substances were adulterants. Thus in the addition of lactose to frozen eggs or dried eggs or of lactic acid to preserve the red color of added beet juice in horseradish, the lactose or lactic acid may be considered adulterants if they have been added to conceal inferiority. Forbidding their use for such purpose would be a health measure.

The ordinary types of preservatives, e. g., boric acid, benzoates, salicylates, etc., may not be toxic except in large quantities. Nevertheless, their use is prohibited except when declared and in many cases even when declared because first, they may conceal damage or inferiority as in the case of sulfites in meat or fruit, secondly, they may be unknown to the consumer as in the case of undeclared benzoates in ketchup and thirdly, if maximum limits are not enforced even when the preservatives are allowed a consumer may at any one meal or time have preservative in every item in this meal and the cumulative effect at that time may be dangerous.

Preservatives in general may be classified under 3 headings:

(1) Inorganic substances such as borates, iodates, free chlorine, hypochlorites, fluorides, peroxides, etc.,
(2) Organic substances such as formaldehyde, benzoates, salicylates, formic acid, p-hydroxybenzoic acid esters, etc.,
(3) Sweetening agents such as dulcin and saccharin.

The function of these sweetening agents is sometimes also germicidal but to repeat they are most often used to conceal inferiority. They will be treated with the organic preservatives as one group. In this chapter general qualitative tests for the aforementioned substances will be outlined. Quantitative methods will be detailed for some of them and references to other methods will be given.

Because of great advances in refrigeration, canning, bottling, cleaning of containers and general handling, the use of preservatives has decreased considerably in the past twenty years. Thus, in recent years, it is seldom that the food analyst finds preservatives in milk, whereas formerly the analysis of milk for preservatives was routine.

However, it must be borne in mind that in times of stress or of food shortage, every effort must be made to conserve food supplies. Thus in 1934, the German Government [1,2] liberalized the use of many preservatives such as borates, benzoates, hydroxy-benzoates, etc., in order to conserve its food supply.

Unscrupulous dealers and manufacturers will have no compunction about the use and non-declaration of preservatives, therefore, it behooves the analyst even though the general use of antiseptics has declined to be on the lookout for such substances. As in the use of color, the use of preservatives very often follows the price scale, that is as the price of food materials rises, the use of preservatives increases.

In some instances, as for example benzoates in cranberries, sulfur dioxide in horseradish, or boric acid in fruits the preservative may be a natural constituent. In these cases, quantitative determinations have to be made and only if the preservative content exceeds the known natural limits may the analyst conclude that preservative has been added.

Boric Acid and Borates

Boric acid and turmeric react to yield a characteristic red color. The production of this color forms the base of the following test for borates as it did for turmeric in Chapter III.

Prepare strips of turmeric paper by soaking filter paper in an alcoholic extract of ground turmeric. Allow the paper to dry spontaneously and then cut into strips.

If the sample is a solid, prepare a paste of the material with water.

[1] *Food Manuf.* 9, 102 (1934).

[2] *Analyst* 59, 348 (1934).

Otherwise use the sample as is and add 7 cc. of hydrochloric acid for every 100 g. of sample used. Stir vigorously, and dip into the mixture a piece of the prepared turmeric paper. Allow the paper to dry spontaneously. If a red color develops which is changed by ammonium hydroxide solution to a dark blue green and back again to red by acid, boric acid is present.

Quantitative Determination[3]—The volumetric method for the determination of boric acid, after ashing, in foodstuffs is liable to considerable errors when applied to samples of food containing large proportions of fat, or phosphates or small proportions of boric acid such as those found naturally in certain fruits. The removal of phosphate requires great care and the results obtained by titration are sometimes vitiated by the accidental presence of phosphates or carbonates. Avoidance of these errors may be made by making use of the ready esterification of boric acid with methyl alcohol, distillation of the methyl borate formed and final titration after the addition of mannitol.

The acidified ash is boiled with methyl alcohol in a 300 cc. Kjeldahl flask, and the vapors are carried over into a vertical, double surface condenser to which the Kjeldahl is inclined at a 30° angle. The exit tube of the condenser is elongated and dips below the surface of alkaline methyl alcohol in a conical flask. The flask stands on a tripod and gauze and is strongly heated by a burner. It is closed by a rubber stopper which carries, besides the condenser exit, another wide glass tube passing through a second hole in the stopper of the Kjeldahl flask down into the methyl alcohol. The ascending limb of this tube must be lagged, which may be conveniently done by enclosing it in a rubber tube. Immediately above the stopper of the conical flask is a side tube sealed on to the condenser outlet. This runs up close to the condenser and carries a trap bulb and a thistle funnel. This tube serves to maintain atmospheric pressure inside the apparatus, to prevent possible losses through bumping in the receiver flask, and to make possible the washing out of the lower part of the condenser outlet.

From 40 to 50 g., or less when large amounts of boric acid are anticipated, is moistened with 10 cc. of 2 *N* sodium hydroxide solution. If much fat is present, it should be removed by washing with petroleum ether, decanting the petroleum ether layer, repeating the process until nearly fat free. The water, and, if petroleum ether was used, the

[3] Alcock, *Analyst* **62,** 522 (1937).

petroleum ether are evaporated on a steam bath, and the sample is ashed. There is no need to burn away all the carbon. The ash is transferred to the Kjeldahl flask with as little water as possible. The dish is finally washed with a few cc. of dilute sulfuric acid, and the ash in the flask is dissolved in a further quantity of acid by warming. This removes most of the carbon dioxide and dissolves the lumps of ash. Methyl red is added and 30 per cent sodium hydroxide solution is run in until the color changes to yellow. The liquid is concentrated to one or two cc. over a burner, with continuous stirring. After cooling, 60 cc. of methyl alcohol and 1 cc. of methyl red solution is added. Sulfuric acid is then dropped in until, after shaking, the solution is strongly acid to the indicator. The flask is then attached to the apparatus, as is the receiving flask containing 0.5 cc. of *N* sodium hydroxide solution and a few drops of phenolphthalein solution. The Kjeldahl flask is heated and, when sufficient alcohol has collected in the receiving flask, that too, is strongly heated, so that the vapor bubbles vigorously through the acid liquid in the Kjeldahl flask. The flame beneath that flask is then adjusted so that 15 to 20 cc. of methyl alcohol remain. If during the distillation the color of the phenolphthalein in the conical flask is discharged, a further 0.5 cc. of the sodium hydroxide solution is added through the thistle funnel.

After 30 minutes the flame beneath the Kjeldahl flask is replaced by a beaker of cold water, and as much of the methyl alcohol as possible is distilled up into the Kjeldahl flask and may be recovered subsequently. The receiving flask is removed, and the condenser tube, both inside and out, is washed into it with water. The remaining methyl alcohol is boiled off, and the solution is then made just acid to methyl red with 0.1 *N* sulfuric acid and boiled for a further five minutes to remove carbon dioxide. After cooling, the acidity is re-adjusted with 0.05 *N* sodium hydroxide solution until it is just not acid to methyl red. More phenolphthalein is added, and, after addition of 1 g. of mannitol, the titration is carried to the phenolphthalein change. A blank determination is made with water in place of a distillate. This result is usually of the order of 0.1 cc. of 0.05 *N* sodium hydroxide solution. One cc. of sodium hydroxide solution is equivalent to 0.0031 g. boric acid. A volume of glycerol, neutral to phenolphthalein, equal to the volume of the solution to be titrated may be substituted for the mannitol.

Sulfur Dioxide and Sulfites

The presence of sulfites as such in a material is not easy to detect qualitatively within the foodstuff because of the possible presence of

other reducing agents. However, the following rapid method for the determination of sulfur dioxide, modified from that of Alesi,[4] overcomes some of these difficulties. This method is based on the bleaching action of sulfur dioxide on iodine. The sulfur dioxide is carried over the iodine in a foam and current of gas caused by carbon dioxide.

Place 20 to 50 g. of the food material rubbed up to a paste with 20 cc. of water, if solid, in a wide mouth 8 ounce bottle. Add 20 cc. of a solution containing 2 per cent sodium hydroxide and 3 per cent sodium carbonate. Then acidify with 30 cc. of hydrochloric acid (1 : 1). When the foam has decreased, suspend in the bottle a piece of starch-potassium iodide paper slightly blued with iodine vapor or by dipping into a dilute solution of chloramine-T. The starch-potassium iodide paper is suspended by inserting it into a slit made in a cork, provided with a vent, that fits the wide mouth bottle. If sulfur dioxide is present the paper is decolorized. Some measure of the amount of sulfur dioxide present is obtained by the rapidity with which the starch-iodide paper is decolorized.

Rapid Quantitative Method—The determination of sulfur dioxide or sulfites is generally made on a quantitative basis. The following method is comparatively short, practical and does away with the sometimes unnecessary refinements of distillation in an atmosphere of carbon dioxide or some other inert gas.

Exactly the same set-up is used as that needed for a Kjeldahl distillation. Transfer 50–100 g. of the material, comparatively accurately weighed, to an 800 cc. Kjeldahl flask. Add 250 cc. of water and 15 cc. of 20 per cent phosphoric acid to make certain that the mixture is distinctly acid. The flask is now connected by means of a trap to a condenser equipped with an adapter dipping into and under the level of a dilute solution of iodine in a beaker. Distill over about 150 cc. If the iodine is decolorized by the liberated and distilled sulfurous acid, sulfites are present. It is possible that other materials may cause the decolorization. Add more iodine solution immediately and continue the distillation until no further decolorization takes place. Care must be taken that the distilled solution does not foam over or that the iodine solution is sucked back. Remove the beaker, wash the adapter, catching the washings in the beaker and boil off the excess iodine. Make acid with hydrochloric acid and add 10 per cent barium chloride solution until no further precipitation takes place. If no precipitate is formed, sulfites

4 Alesi, *Ind. ital. conserve aliment.* **11**, 47 (1936).

are absent. If a precipitate is formed, digest on a hot plate for an hour or leave in a warm place over night and then filter through ashless filter paper. Wash thoroughly with water. Transfer the filter paper and precipitate to a weighed quartz or platinum crucible previously heated to the same temperature as that to which the ash will be heated and burn to a white ash in a muffle. Remove from the muffle, place in a desiccator and weigh when cool. The additional weight is due to barium sulfate. Calculate the amount of sulfur dioxide present and report in parts per million of the original sample.

Care must be taken in the interpretation of results, especially for low amounts of sulfur dioxide. If any doubt arises a more precise determination in an oxygen free atmosphere should be made. Such a method is that of Monier-Williams [5] which is applicable in the presence of other volatile sulfur compounds but is not in the presence of nitrates and nitrites.

Monier-Williams Method—Connect an 800 cc. Kjeldahl flask to a sloping reflux condenser, as illustrated in Fig. 36, the lower end of which is cut off at an angle. Pass carbon dioxide from a generator or tank through a sodium carbonate solution. Connect the upper end of the reflux condenser to the bottom of a small flask which is connected in turn with a Peligot tube. One Peligot tube has been found to be sufficient to catch traces of sulfurous acid swept through the small receiving flask. The receiving flask contains 15 cc. of pure neutral 3 per cent hydrogen peroxide and the Peligot tube contains 5 cc. of the same reagent. Hydrogen peroxide generally contains free sulfuric acid. In order to free the peroxide from sulfate, start with 30 per cent hydrogen peroxide, dilute somewhat, and neutralize with barium hydroxide solution, using bromophenol blue solution as indicator. After the reagent has settled in the cold, filter from the barium sulfate and determine its strength by permanganate titration, and finally adjust to a 3 per cent strength. The bromophenol blue indicator in the hydrogen peroxide remains unaffected for some time.

Connect the apparatus, introduce into the flask 300 cc. of water and 20 cc. of hydrochloric acid and boil for a short time in a current of carbon dioxide. Then add the food to be tested, adopting the procedure to the sort of food. Add liquids directly by means of a dropping funnel

[5] Monier-Williams, Ministry of Health, Reports of Public Health and Medical Subjects, No. 43. London (1927).

and solids by rapid transfer directly to the Kjeldahl flask. After introducing the food, boil the mixture for 1 hour or an hour and a half in the case of dried fruits in a slow current of carbon dioxide, stopping the flow of water in the condenser just before the end of the distillation. This will cause the condenser to become hot and drive over the residual traces of sulfur dioxide retained in the condenser. When the delivery tube

FIG. 36. Monier-Williams Apparatus

just above the receiving flask becomes hot to the touch, remove the stopper connecting the delivery tube to the upper end of the condenser.

Wash the contents of the Peligot tube into the receiving flask and titrate the liquid at room temperature with 0.1 *N* sodium hydroxide, using bromophenol blue as indicator. The sodium hydroxide must be standardized with this indicator. Bromophenol blue is unaffected by carbon dioxide and also gives a distinct color change in cold hydrogen peroxide. One cc. of 0.1 *N* sodium hydroxide solution is equivalent to 3.2 mg. of sulfur dioxide, so that titration of small quantities of sulfur

dioxide requiring less than 0.5 cc. of sodium hydroxide is not accurate. A gravimetric determination may be made after titration, the precipitation of barium sulfate being carried out at room temperature. After allowing the supernatant liquid to settle, filter, and wash the residual barium sulfate 3 times by decantation with boiling water. Determine a blank on the reagents, both by titration and gravimetrically and correct the results accordingly.

Determination of Sulfites in the Presence of Nitrates and Nitrites.—Sulfur dioxide may not be recovered if nitrates or nitrites are present due to the oxidizing action of the nitrates and nitrites in the presence of the hot dilute acid necessary for the Monier-Williams method.[6] To overcome this the nitrate and nitrite may be reduced while leaving the sulfites unattacked. This may be done as in the following method.

Five g. of hydrazine sulfate are dissolved in 100 cc. of hot water and transferred to the Kjeldahl flask of the Monier-Williams apparatus. The liquid is boiled to expel air, and cooled in a current of carbon dioxide. Twenty cc. of a 20 per cent solution of caustic soda is added through the tap-funnel, if used, and the flask is thoroughly shaken. The caustic soda is followed by a suitable quantity of the liquid or solid to be tested and the contents of the flask is boiled in a current of carbon dioxide for 5 minutes. The flame is then removed from the flask and 10 cc. of phosphoric acid, sp. gr. 1.75, and a small quantity of pumice powder are added through the tap-funnel. The contents of the flask is boiled in the current of carbon dioxide and the sulfur dioxide distilled and collected in hydrogen peroxide in the usual way. In applying this process to brine and meat pickles, it is desirable to dilute the reduced solution with boiled water before the addition of phosphoric acid.

Fluorides

Hydrofluoric acid reacts with glass and therefore can be used for etching glass. This property is the basis of the following test for the detection of soluble fluorides in the absence of silica. Boil 150 cc. of the sample or of an aqueous extract of the sample. Add 5 cc. of 10 per cent potassium sulfate solution and 10 cc. of 10 per cent barium acetate solution. Centrifuge to collect the precipitate of barium fluoride. Filter on a small filter. Transfer to a dish and ash.

Prepare a microscope slide or similar glass plate by cleaning and

[6] Sherratt, *Analyst* 62, 267 (1937).

dipping into a hot mixture of equal parts of carnauba wax or wax of similar melting point and paraffin and allow to cool. Make a characteristic mark through the wax. Place the ash in a small flat-bottomed lead dish and add a few drops of sulfuric acid. Cover with another lead dish having an orifice of about 1 cm. Cover the orifice with the glass plate so that the characteristic mark is directly over the orifice and warm, keeping the glass plate cool so that the wax will not melt. If fluorides are present, in sufficient quantity, the glass will be distinctly etched where the characteristic mark was made. Smaller quantities must be determined by a far more sensitive method to be discussed in the chapter on inorganic determinations, Chapter XVII.

To determine insoluble fluorides and soluble fluorides in the presence of silica, a somewhat different procedure must be followed. The hydrogen fluoride formed reacts with the silica to yield gaseous silicon fluoride which reacts with water to form gelatinous silica and hydrofluosilicic acid.

Add enough lime water to about 200 g. of the sample to make it alkaline and evaporate to dryness. Ash and leach the ash with water and acetic acid to decompose carbonates. Filter, re-ignite the residue and re-extract with acetic acid, (1 : 1). The filtrate contains any boric acid that might be present and which may be tested for as detailed above. The residue contains calcium fluoride and silicates.

Ash the filter and residue in a platinum crucible. Add some precipitated silica and 1 cc. of sulfuric acid. Cover with a watch glass plate from which a drop of water is suspended. Heat on a bath for an hour at 70–80° C. If a gelatinous precipitate of hydrated silica due to the reaction between the silicon fluoride and water is formed, fluorides are present.

$$2CaF_2 + 2H_2SO_4 + SiO_2 \rightarrow 2CaSO_4 + SiF_4 + 2H_2O$$

$$3SiF_4 + 4H_2O \rightarrow Si(OH)_4 + 2H_2SiF_6$$

If both borates and fluorides are present, the original substance probably contained a fluoborate. If the gelatinous precipitate is given without the addition of silica, fluosilicate probably was present in the original sample.

Iodates

The literature of recent years mentions the use of calcium and potassium iodate as a preservative. A simple method for the detection of these

substances is based on the iodide-iodate reaction. Make an acid serum of the sample by the addition of sufficient dilute hydrochloric acid to an aqueous mixture or solution of the sample to make it slightly acid and filter. Then add to the filtrate potassium iodide and starch and allow to stand for a few minutes. The following reaction runs with the formation of free iodine and the subsequent production of blue color in the starch.

$$KIO_3 + HCl \rightleftarrows HIO_3 + KCl \text{ and } KI + HCl \rightleftarrows HI + KCl$$
$$HIO_3 + 5HI \rightarrow 3H_2O + 3I_2$$

An alternative method is a variation of the famous Landolt reaction. Again an acid serum of the sample is made and starch is added. Then a solution of a sulfite is added drop by drop until a slight excess of sulfite is present. When the excess of sulfite is used up, the formation of a blue color indicates the presence of an iodate. This color is formed by the following series of reactions.

$$2HIO_3 + 5H_2SO_3 \rightarrow I_2 + 5H_2SO_4 + H_2O$$
$$I_2 + H_2SO_3 + H_2O \rightarrow 2HI + H_2SO_4$$
$$HIO_3 + 5HI \rightarrow 3H_2O + 3I_2$$

Only when the excess of sulfite is used up, will the blue color with the starch appear.

Free Chlorine

The presence of free chlorine in a substance may be detected in the following manner. To an acid solution of the substance add a solution of potassium iodide and starch solution. The chlorine displaces the iodide, forming free iodine. A blue color indicates the presence of free chlorine. Starch-iodide paper serves equally well at times, especially if the color is likely to be masked by the material being analyzed.

Oxidizing Agents

Hypochlorites, nitrites, nitrates, etc., may be detected by the diphenylamine reaction. Dissolve 1 g. of diphenylamine in 100 cc. of sulfuric acid. Add a few drops of the reagent to a small portion of an aqueous extract of the material in a porcelain dish. An intense blue color at the junction shows the presence of an oxidizing agent.

A more sensitive test is obtained if diphenylbenzidine is used instead

of diphenylamine. Wood, Illing and Fletcher [7] found that this reagent will detect 0.1 ppm. of nitrite in milk.

Free chlorine in small amounts is not detected by the diphenylamine reagent and should be tested for as directed above.

Peroxides

The use of hydrogen peroxide, in particular, and peroxides, in general, caused a great deal of interest a number of years ago when their presence in chocolate flavored drinks was demonstrated. The usual test for peroxides in milk is to pour 10 drops of a solution of vanadic pentoxide or of ammonium vanadate in 100 cc. of concentrated sulfuric acid down the side of the test tube containing the sample. A reddish color indicates the presence of peroxides.

In the case of chocolate flavored drinks, or chocolate flavored milk, this color is easily masked. The following test devised by Jacobs [8] overcomes this difficulty. To 10 cc. of the sample add 1 cc. of a 40 per cent solution of trichloroacetic acid and after allowing to stand for 5 minutes, filter. To 3 cc. of the serum produced, add 2–5 drops of a solution of titanium oxide in concentrated sulfuric acid. A fairly persistent yellow color indicates the presence of peroxide. This may be confirmed by adding to another 3 cc. portion of the trichloroacetic acid serum, a few drops of vanadium pentoxide-sulfuric acid solution, 1 g. of vanadium pentoxide dissolved in 100 cc. sulfuric acid (1 : 3). A fairly persistent reddish color verifies the presence of peroxides.

Contrary to the general conception, peroxides do not disappear readily on addition to milk products. Thus, the author obtained positive tests for peroxides as late as 1 month after its addition to samples by the above test.

Organic Preservatives

There are hundreds of organic substances which have been suggested as food preservatives. Many of them are sold under trade names, as for example, Nipasol, Nipagin, Moldex, etc. The following are some of the more common trade names and their organic name: [9]

Nipagin, Solbrol, Moldex are p-hydroxybenzoic acid methyl ester

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[7] Wood, Illing, and Fletcher, *Analyst* 58, 149 (1933).

[8] Jacobs, unpublished data (1936).

[9] Jansen, *Chem. Weekblad* 33, 1 (1936).

Nipasol is p-hydroxybenzoic acid propyl ester

Nipacombin-A is a 6:4 combination of propyl and ethyl esters of p-hydroxybenzoic acid, sodium salt

Moldol is a mixture of sodium benzoate and benzoic acid

Paragerm is methyl-propyl-diphenol-p-oxybenzoate

Microbin is the sodium salt of p-chlorobenzoic acid

Many of these organic materials have actually been used in food materials and some of them are permitted in some countries, as for example Germany, although no extensive work has been performed to establish their harmlessness. Fortunately they need be used in such small quantities that this danger is lessened although not eliminated. The price and efficiency of these organic materials limits their use to a very small group.

The general means of detecting organic preservatives and sweetening agents falls into two main classes:

First, if volatile, they may be distilled and subsequently identified, for example, formaldehyde and formic acid.

Secondly, if soluble in a water immiscible solvent, they may be extracted by that solvent and again subsequently identified, for example, benzoic acid, salicylic acid, saccharin and many others.

Formaldehyde

There are many well known tests for formaldehyde. Some of these may be performed directly on the food sample whereas others can only be made on an alcoholic extract or on the distillate from phosphoric acid solution of the food material. Among these tests are the sulfuric acid-ferric chloride reaction of Hehner, the hydrochloric acid reaction of Leach, the phenylhydrazine hydrochloride reaction, the phenylhydrazine hydrochloride plus sodium nitroprusside reaction, etc. The following two tests are not generally given in texts of food analysis. One is a short method, the other is somewhat longer but is far more sensitive.

1) To about 10 cc. of the sample, made acid with at least 7 cc. of hydrochloric acid to 100 cc. of sample or prepared solution or mixture, add 1 cc. of Schiff's reagent, that is fuchsin-sulfite reagent, prepared as directed in the section, "determination of aldehydes," Chapter XIV, and allow to stand. If a pink to deep violet color develops on standing, formaldehyde is present. This test can be performed directly on most food products, however, if the color may be masked, or it is desired to

make the test more sensitive, it should be performed with 10 cc. of a distillate.

2) *Weinberger*[10] *Dimetol Method.* Prepare a 5–10 per cent solution of dimethylcyclohexanedione in alcohol. This reagent is also known as dimetol and dimethylhydroresorcin. To an aqueous solution of the aldehyde add sodium chloride and neutralize or make faintly acid with acetic acid. Add a few drops of the reagent to the cold solution and stir vigorously. If formaldehyde or any other aldehye is present a precipitate will form. Filter off the precipitate and recrystallize from hot water or alcohol and determine the melting point. Dimetolformaldehyde melts at 187° C.

Formic Acid

Steam distill about 50 g. of the sample made acid with 15 cc. of 20 per cent phosphoric acid until about 200 cc. of the distillate has been collected. Test the distillate for formaldehyde by one of the above tests. If formaldehyde is absent, add to another portion of the distillate sulfuric acid (1 : 4) and some magnesium filings. The hydrogen produced will reduce the formic acid, if present, to formaldehyde which may then be detected by one of the above methods.

Ether Extractive Preservatives, Copper Sulfate Method[11]

This method is a rapid, simple procedure for the detection of ether extractive preservatives. It avoids the formation of troublesome emulsions that are often encountered in other methods. Interfering substances, such as proteins and fats, are removed by precipitation with copper sulfate solution and sodium hydroxide solution. The preservatives are then extracted with ethyl ether and subsequently identified.

To 50 g. of the sample in a beaker, if pasty or solid, add 50 cc. of water and mix thoroughly. If the sample is liquid, use 100 cc. directly. Now add 5 cc. of 10 per cent sodium hydroxide solution and stir well. Add 10 cc. of 35 per cent copper sulfate solution, 350 g. $CuSO_4.5H_2O$ dissolved in water and made to a liter, and stir well. Place on a hot plate and bring to a boil. Stir vigorously and filter at once through a filter directly into a separatory funnel. Cool under running water. Add 5 cc. of hydrochloric acid and mix. Add 75–100 cc. of ethyl ether and shake. Allow to separate. Draw off the water layer and discard. Wash

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10 Weinberger, *Ind. Eng. Chem., Anal. Ed.* 3, 365 (1931).

11 Braverman, personal communication (1930).

the ether layer three successive times with 5 cc. portions of water, drawing off each washing before the addition of the subsequent one. Transfer the ether layer to an evaporating dish, and evaporate off the ether. If no crystalline residue remains, preservatives are absent. If a crystalline extractive remains, it may be benzoic acid, salicylic acid, methyl p-hydroxybenzoic acid and analogues, furoic acid, saccharin, dulcin, etc., or some fruit extractive.

Benzoic acid may be verified by its odor, crystalline appearance, by subliming the residue, by the formation of ethyl benzoate with its characteristic fruity odor on boiling an alcoholic solution of the sublimate with concentrated sulfuric acid, by the formation of a flesh colored precipitate on the addition of ferric chloride solution to an aqueous solution of the sublimate.

Salicylic acid may be identified by the formation of a deep violet color with ferric chloride solution. Other substances also give colors with ferric chloride solution but they do not have the shade given by salicylic acid. In case of doubt, apply the Jorissen [12] test. To an aqueous solution of the residue add 4–5 drops of 10 per cent potassium nitrite or sodium nitrite solution, 4–5 drops of acetic acid, and 1 drop of 1 per cent copper sulfate solution. Boil. If a blood red color is produced, salicylic acid is present. Phenol will give the same reaction.

Saccharin may be verified by its sweet taste and by transforming it to salicylic acid, which may then be identified as directed above. To do this, transfer the residue by means of ether to a nickel dish and evaporate off the ether. Add 10 cc. of 10 per cent sodium hydroxide and evaporate to dryness. Bake for an hour or so and fuse cautiously over a Bunsen flame. Leach with water and transfer to a separatory funnel. Make acid with hydrochloric acid. Extract with ether and wash as directed above. Transfer the ether layer to an evaporating dish and evaporate off the ether. Test the residue for salicylic acid. If the test for salicylic acid was negative before fusion and positive after fusion, saccharin was present in the original sample.

To identify other preservative substances, which it might be said in passing, are very seldom used in the United States, probably because of price and because they are looked upon with disfavor, purify the ether extract either by sublimation or by recrystallization from a suitable solvent. Determine the melting point [13] and the crystalline structure by

12 Jorissen, *Bull. acad. roy. sci. let. Belg.* [3] 3, 259 (1882).

13 Jansen, *Chem. Weekblad* 33, 1 (1936).

means of a microscope. If these tests are insufficient to identify the compound, the analyst may proceed as directed under the subsequent section, "separation of organic preservatives and sweetening agents" which may give a clue to the identity of the substance. Otherwise the analyst must proceed according to a systematic method of organic analysis, as is described in Mulliken, "Identification of Pure Organic Compounds," or some similar text.

Saccharin

Saccharin or benzoylsulfonimide may be estimated quantitatively by the Giuseppi [14] method, in which the imide group is hydrolyzed by means of hydrochloric acid and is subsequently determined by Nessler's reagent. [See Chapter I.] Prepare an aqueous solution of the material, non-alcoholic beverages may be used directly, and place 50 cc. in a separatory funnel. Add 2 cc. of hydrochloric acid and then extract with two successive portions of ether. Preferably, place the 50 cc. of the sample in the bottom flask of a Jacobs-Singer separatory flask, Fig. 11, add 2 cc. of hydrochloric acid, stopper with the upper flask, add water to the connecting joint and then extract with two successive portions of ether. Decant the ether layers into a separatory funnel and wash with 5 cc. of water acidified with 1 drop of hydrochloric acid. Separate the ether layer and evaporate to dryness. Add to the residue 5 cc. of water and 6 cc. of hydrochloric acid and evaporate to 1 cc. on a hot plate. Repeat this process once more Transfer to a 50 cc. volumetric flask with the aid of ammonia free water. Dilute a suitable portion of this solution to 35 cc. in another 50 cc. volumetric flask, add 2.5 cc. of Nessler's reagent, make to volume and read in a colorimeter against a standard prepared from ammonium sulfate. Exactly 0.3613 g. of the anhydrous ammonium sulfate is equivalent to 1 g. of saccharin, insoluble form, and 1.39 g. of the sodium salt crystallizing with 2 molecules of water of hydration.

Lactic Acid

It has been mentioned in the foregoing that lactic acid is sometimes used as a preservative. It is also worth while to note that lactic acid has been recommended in place of vinegar in a number of food products. A modification of a method used by Palm [15] may be used for its identifi-

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[14] Giuseppi, *Z. Nahr. Genussm.* **18,** 577 (1909).

[15] Palm, *Z. anal. Chem.* **33,** 16 (1894).

cation. Place an aliquot of an aqueous solution or mixture of the sample in the lower section of a Jacobs-Singer separatory flask. Make acid with sulfuric acid and then stopper with the upper section. Add water to the connecting joint and then extract with 3 successive portions of ethyl ether. Plural separatory funnels or a liquid-liquid continuous extraction device may also be used. Decant the successive ether layers into an evaporating dish and evaporate to dryness. Take up the residue in a small portion of water and transfer to a test tube. Add 1 cc. of 10 per cent neutral lead acetate solution and filter through a small filter. Add 1–2 drops more of 10 per cent neutral lead acetate solution and if no precipitate forms add a few drops of alcoholic ammonia. If lactic acid is present, it will precipitate as a heavy granular precipitate of basic lead lactate, $3PbO.2C_3H_6O_3$. If a precipitate forms on the further addition of neutral lead acetate solution, more need be added and the mixture filtered again before the addition of the alcoholic ammonia.

Abrastol

Abrastol, asaprol, is the calcium salt of β naphthol-α-sulfonic acid. Sangle-Ferriere [16] recommends the following method. Boil 200 cc. of the sample with 8 cc. of hydrochloric acid for an hour under a reflux condenser. This treatment converts the abrastol to β naphthol. Transfer the refluxed sample to a separatory funnel and extract with 10 cc. of chloroform. Draw off the chloroform into a test tube, add a few drops of 0.5 N potassium hydroxide solution and place in a boiling water bath. The formation of a deep blue color which changes to green and then to yellow indicates the presence of β naphthol, which in turn indicates the presence of abrastol.

Sinibaldi [17] uses the following procedure. Make 50 cc. of the sample alkaline with a few drops of ammonium hydroxide solution and extract with 10 cc. of amyl alcohol, adding ethyl alcohol if an emulsion forms. A Jacobs-Singer separatory flask may be used conveniently. Decant the amyl alcohol, filter if turbid, and evaporate to dryness. Add to the residue 2 cc. of nitric acid (1 : 1), heat on a water bath until half of the liquid is evaporated, and transfer to a test tube with the addition of 1 cc. of water. Add about 0.2 g. of crystallized ferrous sulfate and an excess of ammonium hydroxide solution, dropwise with constant shak-

[16] Sangle-Ferriere, *Compt. rend.* 117, 796 (1893).
[17] Sinibaldi, *Mon. sci.* 7, 842 (1893).

ing. If the resultant precipitate is of a reddish color, dissolve it in a few drops of sulfuric acid, and add crystallized ferrous sulfate and ammonium hydroxide as before. As soon as a dark colored or greenish precipitate is obtained, introduce 5 cc. of 95 per cent alcohol, dissolve the precipitate in sulfuric acid, shake well and filter. In the absence of abrastol a colorless or light yellow liquid is produced, while a red color is produced in the presence of 0.01 g. of abrastol.

Separation of Organic Preservatives and Sweetening Agents

According to Fischer,[18] the following method may be used to separate and identify some of the more important organic preservatives and sweetening substances. These substances are Nipagin, ethyl p-hydroxybenzoate, Nipasol, benzoic acid, o-chlorobenzoic acid, salicylic acid, cinnamic acid, p-hydroxybenzoic acid, dulcin, p-chlorobenzoic acid and saccharin. The extraction for all of these materials is carried out as directed in the preceding sections of the chapter with ethyl ether; the ethyl ether extract is then shaken out with an aqueous alkali solution. The dulcin remains in the ether layer; all the other substances go into the aqueous alkaline layer. Separate the aqueous layer and acidify. Shake out thoroughly with petroleum ether. The petroleum ether extract contains all the aromatic preservatives except p-hydroxybenzoic acid and saccharin, which are insoluble in petroleum ether.

These two substances may be removed from the water layer by means of ethyl ether. The solubility of o-chlorobenzoic acid in petroleum ether is small, but the repeated use of this solvent removes the acid quantitatively. Dulcin is recovered from the original ether layer. It is redissolved in hot water, filtered, and again extracted with ether and recovered by evaporation of the ether extract.

The other ether and petroleum ether extracts are evaporated and the residues are sublimed. The identification of the crystals obtained follows by determinations of the melting point. The p-hydroxybenzoic acid will sublime more easily than will saccharin.

In a simple mixture of benzoic acid and saccharin, the benzoic acid may be separated from the saccharin by subliming the benzoic acid by heating on a water bath. The saccharin remains as a residue.

An alternative method of procedure is to dry the ether extract, obtained as described in the preceding sections, over anhydrous sodium

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[18] Fischer, *Z. Untersuch. Lebensm.* 67, 161 (1934).

sulfate and then evaporate the solvent below 40° C. The extract is then sublimed in an apparatus consisting of a long aluminium plate,[19] which is heated at one end, while at intervals along the plate are depressions in which portions of the substance are placed and covered by a watch glass. A thermometer near each depression registers the temperature at this point.

P-hydroxybenzoic acid methyl ester gives a red color with Millon's reagent in warm acid solution. It sublimes at about 70° C. If it is necessary to cool the receiver during this operation, the crystals obtained are in the metastable condition and melt at 110° C., the stable type melts at 126° C. Saponification with 5 cc. of 2 per cent potassium hydroxide solution produces methyl alcohol, which may be separated by distillation and subsequently identified and estimated as described in Chapter XIV.

P-hydroxybenzoic acid propyl ester and ethyl ester have melting points of 97° and 116° C., respectively. The sublimate may be saponified by boiling for 1 hour with 2 cc. of 10 per cent potassium hydroxide solution and 4 cc. of water under a reflux condenser. Then 4 cc. is distilled and the alcohols in the distillate may be identified by the tests detailed in Chapter XIV. The melting point of the combination of acids produced from Nipacombin-A, the sodium compound of a 6:4 mixture of the propyl and ethyl esters, is 95° C.

P-hydroxybenzoic acid sublimes at 135° C. and has a melting point of 213 to 214° C. Copper sulfate produces small, bright blue crystals when added to the warm acid.

P-chlorobenzoic acid sublimes at 95° C. and melts at 236° C. The ortho compound sublimes at 75° C. and melts at 142° C. A mixture of the extract heated, on a water bath for 20 minutes, with 0.25 cc. of sulfuric acid and a crystal of potassium nitrate followed by the addition of 2 cc. of ammonia water and then 1 cc. of 2 per cent solution of hydroxylamine hydrate yields a green color at the junction of the liquids, if this compound is present.

Cinnamic acid melts at 133° C. and sublimes at 90° C. To identify, make the ether extract alkaline, evaporate and re-extract with acidified ether in the presence of a little alcohol to prevent emulsification. Wash the extract 3 times with water and shake with 0.33 *N* potassium hydroxide solution. Remove the ether from the separated water layer by warming. Add a 1 per cent solution of potassium permanganate when the solution

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[19] Jansen, *Chem. Weekblad* 33, 1 (1936).

is cool, and the benzaldehyde produced, may then be recognized by its odor. The reaction is sensitive to 1 mg. of cinnamic acid, but is lower if the sodium salt is used. The purified acid may be estimated by solution in a known amount of 0.1 *N* sodium hydroxide, the excess of which is back-titrated with hydrochloric acid. The benzaldehyde produced may be confirmed by the following reactions: 1) To the solution containing the benzaldehyde add 1 drop of a solution of phenol and 2 cc. of sulfuric acid. A hard resinous mass is produced on warming. Cool the mixture, dilute with 10 cc. of water, make alkaline with 20 per cent potassium hydroxide solution, benzaldehyde gives a violet color which may be extracted by shaking with acidified ether. 2) To the oxidized liquid containing the benzaldehyde, add twice its volume of a solution of dimethylaniline in sulfuric acid, warm the mixture to 150° C., and dilute with an equal volume of water. Malachite green separates on the addition of potassium dichromate and sodium acetate.

Anisic acid is converted to p-hydroxybenzoic acid and methyl iodide by the action of hydriodic acid. It may be separated from p-hydroxybenzoic acid by extraction with chloroform, in which only anisic acid is soluble.

REFERENCES

Woodman, "Food Analysis," McGraw Hill (1931).
Leach-Winton, "Food Inspection and Analysis," Wiley (1920).
Methods of A. O. A. C. (1935).
Mellor, "Modern Inorganic Chemistry," Longmans (1927).
Thurston, "Pharmaceutical and Food Analysis," Van Nostrand (1922).
Mulliken, "Identification of Pure Organic Compounds," Wiley (1916).

CHAPTER V

METALS IN FOODS

THE determination of metals in foods resolves itself into the problem of determining those metals in the presence of organic material. These procedures present, therefore, no greater difficulty than such estimations ordinarily present. In general, the organic material must be destroyed or removed in some manner before the estimation of the metal is made, and then the usual qualitative or quantitative method applied. Sometimes it is possible to run a procedure for a metal in the presence of organic material without great loss in sensitivity, for example, the Reinsch test for arsenic.

A text of this type cannot go into an exhaustive survey of methods for metals. Furthermore, the food analyst is mainly interested in those metals which are harmful and may come into contact with foodstuffs because of

1) the use of insecticides or fungicides, or

2) through use for a fraudulent purpose, such as the use of lake dyes, mineral pigments, or to give tang; for example, the use of potassium aluminium sulfate in pickles; or to increase the shade of color, for example the addition of copper or zinc salts to intensify the color of chlorophyll; or

3) by contact during a manufacturing process or because of storage in a container; or

4) by accident; or

5) with criminal intent.

His interest is further conditioned by the factor that the presence of anything more than a trace of metal is generally illegal and only approximate quantitative analysis need be made where comparatively large quantities of metal are present.

The food analyst is interested in the following metals: arsenic, lead, mercury, copper, zinc, chromium, cadmium, antimony, aluminium, manganese, tin, nickel and, rarely, thallium. The methods for some of these metals will be detailed and procedures for the others will be outlined. Selenium, sodium, potassium, iodine and other elements will be discussed

in Chapter XVII, "Inorganic Determinations." Authorities are more or less agreed that iron, aluminium, nickel, chromium, silver and gold are non-toxic; copper, tin and zinc are moderately toxic; and lead, antimony, cadmium, mercury, arsenic and thallium are highly toxic.

The role of arsenic in foodstuffs is of historic importance. The numerous cases of arsenic poisoning in Manchester, England, in 1900, traced to the presence of arsenic in beer made with commercial glucose that was manufactured with contaminated sulfuric acid, brought the subject of metallic contamination in food sharply to the public. In recent years, the use of arsenical, mercurial and lead bearing and other metallic insecticides and fungicides to preserve the growing food supply from destruction by insect and mold pests has increased enormously. The realization of danger of epidemic proportions lying in the use of these metallic insecticides and fungicides without proper removal before the food is sold for consumption has developed a great deal of interest in methods for the detection and determination of very small amounts of these metals. In the opinion of the author some of these lines of investigation, namely the dithizone and 8-hydroxyquinoline methods, are non-specific and should be used and viewed with great care.

Preparation of Ash

There are two general methods for the destruction of interfering organic matter. The first is called the "wet ash" or acid digestion method and the second is ordinary ashing by means of heat with, or without, the aid of an "ash aid" mixture or of an alkaline fixitive for volatile metals such as arsenic or mercury. Acid digestion is to be preferred for arsenic, mercury and tin.

Wet Ash or Acid Digestion—Depending on the type of foodstuff and whether the metallic contamination is throughout the product as might be possible in the case of fish, or whether it is exclusively on the outside as in the case of insecticide on fruits, weigh a representative portion of the product, generally 100 to 200 g., sometimes varying from 5 g. to 5 lbs., according to the metal content, and peel, if possible. Place the weighed portion or the peelings in one or more 800 cc. Pyrex Kjeldahl flasks. Add 50 cc. of nitric acid and then carefully add 20 cc. of sulfuric acid. Heat cautiously so that no excessive foaming takes place. Add nitric acid in small portions until all the organic matter is destroyed.

This point is reached when no further darkening of the solution occurs on continued heating after the production of a clear solution and copious fumes of sulfur trioxide. Cool, add 75 cc. of water and 25 cc. of a saturated solution of ammonium oxalate to aid in the expulsion of nitrogen fumes. Evaporate again to the appearance of sulfur trioxide fumes. Cool, dilute with water, transfer to a 500 cc. or liter volumetric flask, and make to volume. Use aliquot portions for the analyses detailed below.

Ashing with Nitric and Perchloric Acid.—Substances which have an appreciable vapor pressure at the boiling temperature of a mixture of nitric and sulfuric acids are likely to be lost. The possibility of formation of insoluble compounds is also present in that type of acid digestion. The replacement of sulfuric acid by perchloric acid eliminates these difficulties and makes it possible to make a number of different determinations on the same wet-ash. Hot perchloric acid may react violently with organic materials and consequently it is best to control the reaction intensity by pretreating the materials with nitric acid. Great care must always be exercised when using perchloric acid.

Gieseking, Snider and Getz[1] propose the following method: Place a 4 g. sample of the material to be oxidized in a 400 cc. beaker and add 10 cc. of nitric acid (sp. gr. 1.42). Cover the beaker with a watch glass and heat gently until any rapid initial reactions have subsided. Then heat to boiling and boil until the contents of the beaker are almost dry. Remove the beaker from the hot plate and add 10 cc. of dilute nitric acid (1:1) and 10 cc. of perchloric acid (70 to 72 per cent). Replace the cover glass and heat very gently to a low boiling temperature. Avoid superheating. Maintain this temperature until all organic material has been removed from the sides of the beaker and from the solution, which will be indicated by a colorless or slighly colored solution. Remove the cover glass, allow the beaker to cool a few minutes, and wash any adhering salts into the beaker. If the cover glass is washed with perchloric acid, the contents of the beaker need not be cooled.

Evaporate to dryness in a hood at a temperature just below the boiling point. If potassium is to be determined on the residue by a method in which ammonium salts interfere, the ammonium salts may be removed at this point. After the removal of ammonium salts, add 5 cc. of hydrochloric acid (1 : 1) and 10 cc. of water. Heat until all salts are dissolved. Filter into a suitable volumetric flask. Wash the silica residue

[1] Gieseking, Snider and Getz, *Ind. Eng. Chem., Anal. Ed.* 7, 185 (1935).

thoroughly with hot water and make the filtrate up to volume. Aliquot portions of the filtrate may be taken for subsequent analyses.

The method detailed above is particularly adapted for determinations on calcium, magnesium, potassium and phosphorus.

Gerritz[2] suggests an alternative procedure. Place weighed samples of suitable size in 500 cc. Kjeldahl flasks. Add 20–30 cc. of nitric acid and place the flasks in asbestos gauzes over medium flames. Boil the contents gently with frequent mixing until the samples pass into a semi-colloidal solution. The particles of material become swollen and gel-like, then disintegrate, producing a finer suspension or a solution. Experience will indicate at what time this occurs, and the speed of the digestion may be accelerated materially by determining the length of time required by the material being analyzed to reach this stage. Heating to dryness is to be avoided.

Add 10 cc. of perchloric acid to each flask and heat over a free flame. Low flames are needed during the perchloric acid digestion and best results are obtained when just sufficient heat is applied to keep the solution boiling. Only a fine point of the flame should impinge on the flask. Higher temperatures tend to drive off the perchloric acid without materially accelerating oxidation.

When fuming begins, adjust the flame so that only a trace of the perchloric acid fumes reaches the upper region of the Kjeldahl flask neck. The heating is continued until the solution is practically colorless or only a faint yellow remains. The solution is allowed to cool slightly and 50 cc. of water is added. Vigorous boiling occurs which drives out the remaining nitrogen dioxide fumes, leaving a clear solution. The solution is filtered into a volumetric flask, and the Kjeldahl is thoroughly washed with water. When the solution is cool, it is made up to volume and aliquots are taken for analysis.

Ash by Ignition—Weigh a representative portion of the material to be analyzed and transfer to a porcelain dish or casserole of convenient size. This will be from 5 to 200 g. depending on the amount of metal in the food. Add two to 5 cc. of a solution of aluminium nitrate and calcium nitrate, 40 g. of $Al(NO_3)_3.9H_2O + 20$ g. of $Ca(NO_3)_2.4H_2O$ in 100 cc. of water or an excess of lime or magnesia. Dry in a thermostatically controlled oven at 100° C. Char the material, controlling swelling, if any, by playing the flame from a glass jet over the sample. Ash in a

[2] Gerritz, *Ind. Eng. Chem., Anal. Ed.* **7**, 167 (1935).

muffle overnight, if possible, at not over 450° C. If a clean ash cannot be obtained, cool the dish and add more ash-aid solution, that is, aluminium nitrate-calcium nitrate solution, or 2–3 cc. nitric acid, if permissible, dry and re-ash. Do not add nitric acid if an alkaline ash is necessary, as for example, if arsenic is to be estimated. Dissolve in an appropriate solvent when the ashing process is complete, and proceed with one of the methods detailed below.

Heavy Metals

In general, the qualitative demonstration of the presence of heavy metals in foodstuffs can be made by using the group sulfide precipitations. Transfer the wet ash to a beaker, dilute and neutralize. Add 1 cc. of hydrochloric acid (1 : 3) to each 10 cc. of solution and warm to 50° C. Add an equal volume of saturated hydrogen sulfide solution and allow to stand for 10 minutes at 35° C. If no color or precipitate is produced, the following metals may be considered absent in more than traces: silver, arsenic, antimony, tin, copper, mercury, bismuth, thallium and cadmium. Lead may remain behind as $PbSO_4$, lead sulfate, in the wet ash process, hence, if any residue remains, it should be investigated separately with ammonium acetate solution.

If the mixture of sample solution and hydrogen sulfide is now filtered and made alkaline with ammonium hydroxide, any precipitate which forms may be due to one of the following: aluminium, chromium, zinc, manganese, iron, cobalt and nickel. This portion of the test is not of much value because many food materials contain iron, which would, of course, give the test.

Arsenic

Reinsch Test—The Reinsch test is a simple though not very sensitive one. It is based on the deposition of arsenic from solution as a copper arsenide. This test may very often be applied directly without previous destruction of organic material. Place 200 cc. of the liquid food or beverage, or of a mixture of water and the solid food, in a casserole or similar container, and acidify with 1 cc. of arsenic free hydrochloric acid. Then evaporate to one-half its volume. Add 15 cc. more of hydrochloric acid and also a piece of pure burnished copper foil. The liquid is kept simmering for an hour and the water lost by evaporation should be replaced from time to time. If at the end of this time the copper foil remains bright, arsenic is absent. If the copper has a black or brown

deposit, remove it and wash well with water, alcohol, and ether, and dry. Place the foil in a subliming tube and heat over a low flame. If a sublimate is present, examine under a microscope. Arsenic forms tetrahedral crystals in contradistinction to mercury. Antimony, silver and bismuth will also give a deposit, but will not sublime.

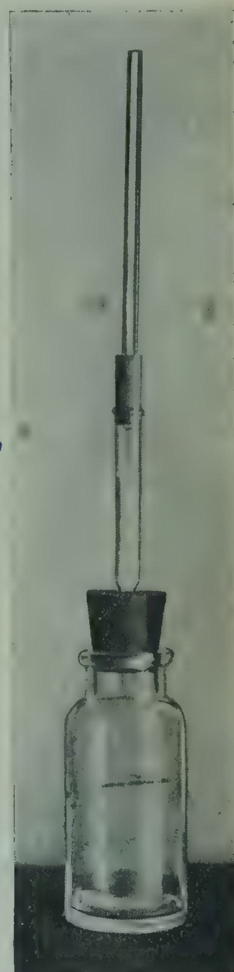

Fig. 37. Gutzeit Generator

Gutzeit Method—The Gutzeit method is based on the liberation of arsine from an arsenic solution. The arsine subsequently reduces mercuric bromide on a prepared strip of paper with the production of stains. The stain, if the method is followed in detail, is proportional to the amount of arsenic.

Prepare a generator, Fig. 37, as follows: Use a 2 oz. wide mouth bottle. Equip the bottle by means of a perforated stopper with a glass tube 1 cm. in diameter and 6–7 cm. long, with an additional constricted end to facilitate connection. Place a small wad of glass wool in the constricted bottom end of the tube and add 3.5 to 4 g. of 30 mesh clean sand. Moisten the sand with 10 per cent lead acetate solution and remove the excess by light suction. The lead acetate is used to remove any hydrogen sulfide that might be generated along with the arsine and thus vitiate results, if permitted to reduce the mercuric bromide. Connect the tube by means of a rubber stopper with a narrow glass tube 2.6 to 2.7 mm. in internal diameter, and 10 to 12 cm. long, and place in this tube a strip of mercuric bromide paper. These strips may be made by cutting paper similar to Whatman No. 40, into strips exactly 2.5 mm. wide and about 12 cm. long. Soak the strips for 1 hour or longer in a fresh 3 to 6 per cent solution of mercuric bromide in 95 per cent alcohol. Dry and use within 2 days. For approximately quantitative work, these strips may be stored in a stoppered blackened tube.

Determine the acid in an aliquot of the solution prepared from the wet ash or ignition described above. Place aliquots not to exceed 30 cc. depending on the amount of arsenic trioxide, 0.01 to 0.03 mg., in the Gutzeit generator. If the aliquot contains only hydrochloric acid, add

sufficient hydrochloric acid to make a total volume of 5 cc. If it contains sulfuric acid, add sufficient 25 per cent, arsenic free sodium hydroxide solution to exactly neutralize it and add 5 cc. of hydrochloric acid; or add sufficient hydrochloric acid to the sulfuric acid in the aliquot to make a total volume of 5 cc. Cool, if necessary and add 5 cc. of potassium iodide solution, 15 g. of potassium iodide dissolved in water and made up to 100 cc., and 4 drops of stannous chloride solution, 40 g. of arsenic free stannous chloride, $SnCl_2.2H_2O$, in hydrochloric acid made up to 100 cc. with hydrochloric acid. Add a piece of activated zinc, 10 to 15 g. or 2 to 5 g. of granulated zinc, center the strip of mercuric bromide paper, and place the tubes in position.

Immerse the apparatus in a water bath kept at 20-25° C. to within 1 inch of the top of the narrow tube and allow the evolution of arsine to proceed for 1 hour or 1.5 hours. Remove the strip and average the length of the stains on both sides in mm. Locate the length of the unknown on a standard graph and read off on the abscissa the quantity of arsenic present. The graph may be made by running known quantities of arsenic by the above method using lengths of stain as ordinates and mg. of arsenic trioxide as abscissa.

$$\text{Grains/lb.} \times 143 = \text{ppm.}$$

$$\text{ppm.} \times 0.007 = \text{grains/lb.}$$

All the reagents used in this determination should be arsenic free. However, as a precaution, it is best to run blanks on the reagents. In some cases the test may be made without previous destruction of organic material but the results obtained are probably only approximate. An aliquot containing 0.02 to 0.025 mg. of arsenic trioxide is considered optimum for reading the stain.

Antimony

Antimony may get into foods that are cooked or processed in enamelware, the enamel of which was made with antimony compounds, or it may contaminate foods that are covered with antimony bearing tin foil. Acids, such as citric may at times extract sufficient antimony to have an emetic or even more harmful effect. It may be detected qualitatively by the Reinsch and other tests. The Reinsch test is detailed under the section, "Arsenic". It may be estimated quantitatively as directed by Bamford.[3] In this method the antimony is thrown down as antimony

[3] Bamford, *Analyst* 59, 101 (1934).

sulfide and is then determined colorimetrically against standards prepared in a similar manner. The antimony sulfide colloid is stabilized by the use of a solution of gum arabic or ghatti.

A weighed portion of the sample is cut up finely and mixed in a silica dish with sufficient magnesium oxide to give a definitely alkaline reaction. The material is then covered with a saturated solution of magnesium nitrate, $Mg(NO_3)_2$. In general, 35 to 40 cc. of this solution are sufficient for 100 g. of animal matter. The mixture is then heated on a sand-bath, with frequent stirring, until the material has dried, charred and begun to whiten. The charred mass may be crushed with a pestle and heated strongly if necessary over a blowpipe flame. The ash should be white. If not, it may be cooled, mixed with a concentrated solution of ammonium nitrate and reheated till free of nitrates. The ash, when cold, is moistened with water and sufficient hydrochloric acid to dissolve the magnesium oxide and to give a definitely acid reaction.

The solution is diluted with water and treated with hydrogen sulfide. The precipitate is filtered off, washed in the usual way and dissolved in a minimum quantity of hot concentrated hydrochloric acid. This solution is diluted with water, refiltered, and after the addition of 1 cc. of 5 per cent gum arabic or gum ghatti solution per 100 cc. of the liquid to hold the precipitated antimony sulfide in suspension, the solution is made up to a definite volume. Hydrogen sulfide is again passed through the solution and the color produced is compared in a Duboscq or other appropriate colorimeter against a standard of approximately equal concentration.

The standard may be prepared by diluting 1 cc. of a 5 per cent tartar emetic solution, slightly acid and mixed with gum arabic or ghatti to 1 liter. An appropriate aliquot is then treated with sufficient gum solution to give the same concentration as that in the unknown and is made up to definite volume. It is then saturated with hydrogen sulfide and used as the standard.

Lead

Lead bearing sprays and powders are being used extensively as insecticides. Modern procedure determines lead in preference to arsenic because fruit or other food materials so sprayed or coated may be freed more easily from arsenic than lead. Lead is considered far more toxic than formerly and the determination of small amounts is important in biochemical estimations as an indication of lead poisoning. Lead sometimes gets into foods which are processed in lead lined tanks or pipes

or that are stored in leaded containers or in containers that are soldered.

The following method based on the precipitation of lead as the sulfide is a comparatively simple one and is adaptable to all types of foodstuffs. Ash an appropriate amount of the material in a porcelain or silica dish, and after obtaining a clean ash as directed in a preceding section, dissolve the ash in 10 cc. of water and 1 cc. of nitric acid. Filter and make the colorless filtrate alkaline with ammonia. The phosphate precipitate will contain practically all of the lead and the copper will be in the filtrate. Filter, wash well and dissolve the precipitate in 5 cc. of dilute acetic acid. Make up to 50 cc. in a Nessler tube. Add 5 cc. of hydrogen sulfide solution and match the color with standard lead solutions treated the same way. The lead sulfide precipitate may be stabilized by the use of gums, arabic or ghatti, and may then be estimated colorimetrically against a standard as detailed in the method for antimony.

Dithizone Method—With the introduction by Fischer [4] of the dithizone method for the determination of lead, numerous variations of this method have appeared.[5] These methods have the ability of detecting very small amounts of lead and are based on the formation of a red precipitate soluble in chloroform or carbon tetrachloride, when an ammoniacal cyanide solution of dithizone is added to a solution containing lead. The value of the accuracy claimed for some of these methods is open to serious doubt, especially since dithizone is not a specific reagent for lead, but will form colored compounds with 14 other metals. Even in the presence of excess potassium cyanide, stannous tin, bismuth and thallium interfere. The difficulty encountered in removing these interferences does not at times compensate for the ease of some parts of the dithizone method.

Dithizone is the short name for diphenylthiocarbazone. It forms a green colored solution in chloroform. The lead complex has a red color and is soluble in chloroform but is practically insoluble in dilute ammonia, whereas dithizone itself is soluble. Upon these factors, the various methods for the isolation and subsequent determination of lead depend.

The commercial diphenylthiocarbazone must generally be purified before use. Dissolve about 1 g. of the commercial reagent in 50 to 75 cc. of chloroform and filter if insoluble material remains. Shake out in a Jacobs Singer separatory flask with four 100 cc. portions of metal free,

[4] Fischer, *Z. angew. Chem.* 42, 1025 (1929).

[5] Winter, Robinson, Lamb and Miller, *Ind. Eng. Chem., Anal. Ed.* 7, [illegible] (1935).

redistilled ammonium hydroxide solution (1:99). Dithizone passes into the aqueous layer to give an orange colored solution. Filter the aqueous extracts into a large separatory funnel through a pledget of cotton inserted in the stem of a funnel. Acidify slightly with dilute hydrochloric acid and extract the precipitated dithizone with two or three 20 cc. portions of chloroform. Combine the extracts in a Jacobs-Singer separatory flask and wash two or three times with water. Pour off into a beaker and evaporate the chloroform with gentle heat on the steam bath, avoiding spattering as the solution goes to dryness. Remove the last traces of moisture by heating for an hour at not over 50° C. in vacuo. Store the dry reagent in the dark in a tightly stoppered bottle. Make up the reagent solutions for extraction to contain approximately 100, 50, and 10 mg. per liter in redistilled chloroform. A stock solution of dithizone in chloroform containing 1 mg. per cc. will keep a long time and is convenient for use in making dilutions.

Rapid Method for Lead Spray on Fruits—One of these methods is the Vorhes-Clifford [6] rapid method for the determination of lead spray on fruits.

Reagent. Sodium oleate solution. To 45 cc. of 30 per cent sodium hydroxide, in a 1.5 liter beaker add 400 cc. of water. Add slowly while heating and stirring, 90 g. (by difference from a separatory funnel) of oleic acid. Heat the mixture on a steam bath until the soap is entirely dissolved, cool, dilute to 1 liter and filter.

Determination. Weigh 1400 g., equivalent to 10 or more apples or pears, or 350 g. of cherries, pull or cut out the stems and expose the junction of stem and fruit. Trim off the sepals. Allow stems and sepals to fall into a large funnel inserted in the neck of a 500 cc. volumetric flask. To 25 cc. of 30 per cent sodium hydroxide solution in a 600 cc. beaker, add 175 cc. of water and 25 cc. of sodium oleate reagent and bring to a gentle boil. Impale each fruit on a pointed glass rod and immerse in the alkaline solution, rotate slowly until the skin begins to check, remove to the funnel, and rinse with the aid of a wash bottle containing hot nitric acid (1:49) or hot hydrochloric acid (3:97), if arsenic is to be determined also, being careful to flush out the stem and calyx ends thoroughly and allowing the rinse acid to flow over the stems

[6] Vorhes and Clifford, *J. Assoc. Official Agr. Chem.* 17, 130 (1934).

and sepals in the funnel. When all the fruit has thus been treated, cool the alkaline solution and add it through the funnel to the acid solution in the flask. Rinse the beaker and funnel with any remaining acid and with water, using the entire 250 cc. of rinse acid. Cool and make to volume. In a flask place 10 cc. of concentrated nitric acid or hydrochloric acid to conform to the kind of acid used in rinsing. Thoroughly mix the sample solution, withdraw 100 cc. by means of a pipette and add to the acid in the flask, while swirling vigorously. Filter on a rapid filter. If the first portion of the filtrate is cloudy, return to the filter until a clear filtrate is obtained.

Transfer 20 cc. portions of the filtrate to each of three small glass stoppered bottles. First add 10 cc. of the ammonia-citric acid-potassium cyanide solution [dissolve 10 g. of potassium or sodium cyanide, phosphate-free, and 10 g. of citric acid in 500 cc. of ammonium hydroxide (sp. gr. 0.90) and dilute to 1 liter.], to each bottle. To one bottle add 20 cc. of standard dithizone solution, 20 mg. of purified dithizone dissolved in 1 liter of chloroform, and to the other two bottles 20 cc. of clear chloroform. Shake the flasks vigorously for 1 minute. Transfer the contents of the bottles to Nessler tubes and allow the layers to separate. With a tube of clear chloroform backing the sample tube which contains the dithizone and one sample tube containing chloroform backing each of two standard tubes, compare the color in the lower layer of the sample with that of the standards. If the range is exceeded, repeat with a smaller aliquot of the filtrate making up to 20 cc. with the "blank" solution.

The standards for comparison are prepared as follows: Place into each of two 1 liter volumetric flasks, 47.5 cc. of 30 per cent sodium hydroxide. According to the rinse acid, add 100 cc. of concentrated nitric or 104.6 cc. of concentrated hydrochloric acid to each flask. To one of the flasks add 7.27 mg. of lead, from a stock solution containing 2 mg. lead (3.197 mg. lead nitrate, $Pb(NO_3)_2$) per cc. in 1 per cent nitric acid. Mark this flask "standard" and the other "blank". Dilute both solutions to volume and mix. By a combination of the two solutions in suitable proportions the equivalent of any lead load from 0 to 0.02 grain/lb. may be obtained. The following table gives the quantities of "standard" and "blank" to be added to the Nessler tubes for each interval. They are conveniently measured into the tubes by means of a burette.

Grain/lb.	Standard cc.	Blank cc.
0.000	0.0	20.0
0.002	2.0	18.0
0.004	4.0	16.0
0.006	6.0	14.0
0.008	8.0	12.0
0.010	10.0	10.0
0.012	12.0	8.0
0.014	14.0	6.0
0.016	16.0	4.0
0.018	18.0	2.0
0.020	20.0	0.0

Then add to each tube 10 cc. of the ammonia-citric acid-potassium cyanide solution, followed by 20 cc. of standard dithizone solution. Shake vigorously for 1 minute, and allow the layers to separate. The color of the lower layer is used as the standard for comparison.

"Mush" Method—An alternative method for placing lead into solution for analysis is termed the mush method. The food material is run through a lead free sheering food grinder 3 times. Place 200 g. of the material in an 800 cc. beaker, dilute to 300 cc. and add 40 cc. of strong nitric acid. The mixture is brought to a boil and is stirred until the initial foaming ceases and a comparatively smooth mixture results. It is cooled, transferred to a volumetric flask, made to volume and filtered. Place 250 cc. of the filtrate in a separatory funnel and extract with dithizone solution. The lead may then be re-extracted with 1 per cent nitric acid from the dithizone solution and may then be determined by a suitable method.

Wichmann-Clifford Method [7]—The method is based on the electrolytic separation of lead as the peroxide and its titration by iodometric means. The lead is deposited on the anodic, positive pole, by the use of a low electric current. Tin, antimony, bismuth and manganese interfere with the deposition and must, therefore, be removed. Samples are ashed and precipitated with hydrogen sulfide, using copper as a collector for the lead. The sulfides are filtered, washed with hot polysulfide solution, and finally with sodium sulfate solution. The lead and copper sulfides

[7] Wichmann and Clifford, *J. Assoc. Official Agr. Chem.* 17, 123 (1934).

remaining are then dissolved in hot nitric acid, neutralized with ammonium hydroxide, and made up to 2 per cent acid, with nitric acid. Potassium dichromate solution is added, the solution heated, electrolyzed, and the lead deposited as the peroxide, PbO_2. It is then washed thoroughly, and removed from the anode, with a sodium acetate acidic solution. Potassium iodide is added and the liberated iodine titrated with 0.001 *N* sodium thiosulfate solution, using starch as an indicator.

Reagents. 1) Polysulfide Solution: Dissolve 480 g. of sodium sulfide, $Na_2S.9H_2O$ and 40 g. of sodium hydroxide, NaOH, in water. Add 16 g. of powdered sulfur, shake until the sulfur dissolves, filter and dilute to 1 liter.

2) Sodium Thiosulfate: Dissolve 24.85 g. of sodium thiosulfate, $Na_2S_2O_3.5H_2O$ in one liter of carbon dioxide free water. Protect the solution with a soda lime tube and thiosulfate trap and allow it to stand for about two weeks. Prepare approximately 0.001 *N* solution by diluting this reagent with carbon dioxide free water in the ratio of 1:100. Standardize this dilute solution by running known quantities of lead nitrate of the order of 2 to 4 mg. or 0.2 to 0.5 mg. respectively. Prepare the dilute solutions at least every other day, but standardize the solution daily.

3) Standard Lead Solution. Lead nitrate may be recrystallized from water to obtain a reasonably pure salt. Dissolve 20 to 50 grams of C. P. lead nitrate in a minimum amount of hot water and cool with stirring. Filter the crystals with suction on a small Büchner funnel, redissolve and repeat the crystallization. Dry the crystals at 100–110° C. to constant weight. Cool in a desiccator and preserve in a tightly stoppered bottle. The product has no water of crystallization and is not appreciably hygroscopic. Prepare a solution containing 10 mg. of lead per cc. in about 1 per cent nitric acid and from this solution make weaker dilutions as needed. Because lead tends to precipitate, probably as a silicate, from very dilute solutions, the weaker dilutions should not be used over long periods of time.

Method. Prepare an ash of an adequate amount of material as directed in the section, "Ash by Ignition." Add 30 cc. of hydrochloric acid (1:1), and heat to boiling. Filter and wash with hot water into a 250 cc. beaker. If a large quantity of unburnt carbon remains, return the residue and filter into the casserole or ashing dish and re-ash. Rinse the ashing dish and then extract the residue with 20 cc. of hydrochloric-citric acid mixture (1:1), the hydrochloric acid containing 20 per

cent citric acid. Filter the extract into the same beaker. Place a few pellets of sodium hydroxide in the ashing dish and add 1 to 2 cc. of hot water. Allow the syrupy solution to wet the inside of the dish completely and heat until nearly dry. Take up the residue in a little water and run the alkaline wash directly into the filtrate, so as not to redissolve the silica that may be on the filter. Finally rinse the casserole or ashing dish with a few cc. of hot hydrochloric acid (1:1) followed by 1 to 2 washings with hot water.

Cool the filtrate. Add 1 cc. of thymol blue, 40 mg. thymol blue indicator in 100 cc. of water, and add ammonium hydroxide from a burette until the color changes from reddish orange to a distinct yellow, at which the pH is 2.8. To obtain a more accurate pH, add 4 drops of bromphenol blue, 40 mg. bromphenol blue indicator in 100 cc. of water, and continue adding ammonium hydroxide until the color changes through olive green to a purple color, which is obtained at a pH of 3.8 or greater. The pH range, for the precipitation of lead sulfide, without co-precipitation of iron sulfide, is from 2.5 to 3.4. The color of the solution using these indicators is an incipient purple to olive green at this pH. Adjustment to the correct pH point must always be made from the acid side to prevent precipitation of alkaline earths, aluminium, iron, hydrates and phosphates.

Add 5 cc. of copper sulfate solution, 10 g. $CuSO_4.5H_2O$ dissolved in water and made up to a liter, and pass hydrogen sulfide into the cold solution for 3 minutes and filter the sulfides immediately, preferably on a Jena Glass Filter No. 11 GA. This type of filter may be cleaned with sulfuric acid, hydrochloric acid, or 10 per cent sodium hydroxide, followed by reverse flushing with hot water. A light mat of asbestos, should cover the filter, to prevent clogging the filter. Dissolve any tin, antimony or arsenic sulfides from the filter with 5 applications of 5 cc. each of warm polysulfide reagent, prepared as directed above. Wash the filter four times with 3 per cent sodium sulfate solution, 3 g. anhydrous Na_2SO_4 in 100 cc. water.

Dissolve the sulfides retained with 5 cc. of hot nitric acid catching the filtrate in a beaker mounted under the funnel, and rinse the filter thoroughly, with hot water. Boil the solution until the sulfur is coagulated or oxidized. Neutralize the solution with ammonium hydroxide, add 2 cc. of concentrated nitric acid and bring the volume to 100 cc. with water. These volumes are preferable when electrodes $1'' \times 5/16'' \times 5''$ overall are used.

The conditions of electrolysis should be carefully regulated. Low acidity, constant speed of the revolving anode, constant elevated temperature and low current density counteract the interference of phosphates, arsenic and traces of chlorides, and insure the complete deposition of small amounts of lead as the peroxide. Low acidities are essential for the amounts of lead usually found in foods, circa 0 to 10 mg. of lead. Higher acidities may be used for larger quantities of the metal. Low current densities are also essential. High acidities and high current densities promote the production of nitrites which seriously interfere with the determination of small amounts of lead.

Before electrolyzing heat the anode to red heat in a Bunsen burner. Heat the solution to about 75° C. over a gas flame and then add 1 cc. of potassium dichromate solution, 100 g. $K_2Cr_2O_7$ dissolved in water and made up to 1 liter, to suppress nitrite formation. Start the current and adjust to 75 to 80 milliamperes and electrolyze, while anode is revolving at 450 R. P. M. Maintain the temperature between 70 to 80° C. Electrolytic apparatus designed so that all the conditions specified may be controlled are available. Fig. 38 illustrates one of these electrolytic devices.

The time of electrolysis may vary according to conditions. Efficient stirring shortens the time so that with proper equipment 15 minutes is usually long enough. If the speed is not great enough, stir the solution well, and if more than 5 to 10 mg. of lead is expected, increase the time to 20–25 minutes.

If possible arrange to remove the acid by siphoning, at the same time adding water to keep the level of the solution above the deposit on the anode. The acid is entirely removed only when the current drops to zero.

Place the anode in a small flat bottom tube, add 5 cc. of stripping solution, consisting of 5 cc. of acetate mixture (to 20 cc. of a saturated sodium acetate solution add 10 cc. of glacial acetic acid and make up to 100 cc. with water), and 1 cc. of potassium iodide solution, 2 g. KI dissolved in water and made up to 100 cc. Add a few drops of starch solution and titrate the liberated iodine, with 0.001 *N* thiosulfate solution using a micro burette. Using the electrode as a stirrer, sight through the entire depth of liquid to detect the delicate end point, which is reached when the last faint starch iodine color just disappears. A white opal base support aids in ascertaining the end point. If the quantity of lead is high, which may be noted from the dark appearance of the anode, the starch solution should not be added until the end point is approached.

This may be detected when the yellow color of the solution begins to disappear. Calculate the quantity of lead determined from the factor

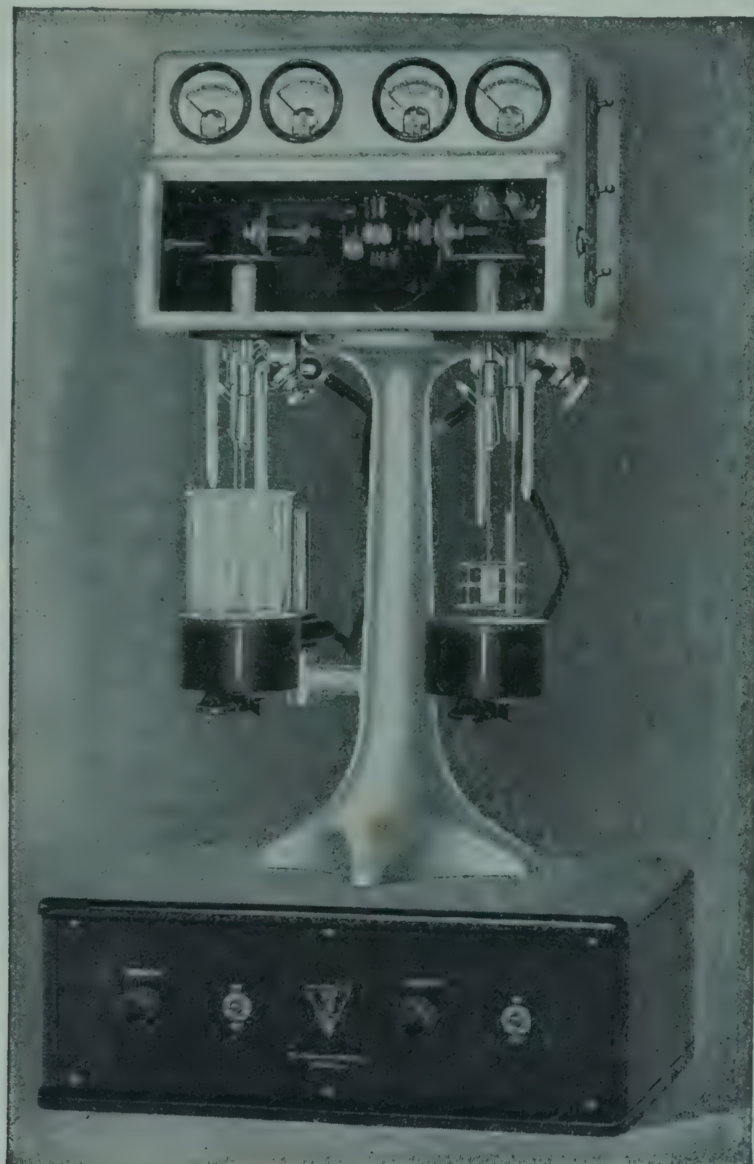

FIG. 38. Electrolytic Apparatus. (Courtesy of Wilkens-Anderson)

of the thiosulfate, previously standardized against pure lead nitrate. The reagents used should be as pure as possible. Every precaution

should be taken to have clean apparatus. As a precautionary measure, it is wise to run blanks with every series of determinations.

Combination Method[8]—As an illustration of a method combining the information of the preceding methods, namely, the mush digestion, dithizone extraction, electrolytic deposition of lead peroxide and iodometric estimation, the following method is appended.

Place 10 g. of the material or other suitable dry weight in a 400 cc. beaker, moisten with water, in order to prevent combustion when heated with nitric acid and add 50 cc. of nitric acid. Allow the contents to stand, with a watch glass over the beaker, until the initial action has subsided. Place the beaker over a flame and boil for about ½ hour or until the material is well mushed. Dilute the mushed solution when cool, transfer to a 300 cc. volumetric flask, make to the mark, allow to stand for 15 minutes, shaking at short intervals, filter, and take a suitable aliquot, say 200 cc. for the analysis. Add to the aliquot in a separatory funnel 20 cc. of citric acid, 20 g. citric acid dissolved in water and made up to 100 cc., and 8 g. of sodium hexametaphosphate. Make this solution just alkaline with ammonium hydroxide, and add 10 cc. of potassium cyanide solution, 10 g. potassium cyanide dissolved in water and made up to 100 cc. Extract with small portions of dithizone solution, 50 mg. dithizone in 100 cc. chloroform, until the color of one portion remains unchanged. Drain the successive chloroform extracts into a smaller separatory funnel containing 20 cc. of ammonium hydroxide (1:99), and shake as a means of washing the chloroform. When the extraction is complete, drain the combined portions of dithizone-chloroform solution, containing the lead, into a 150 cc. beaker and evaporate to dryness over a steam bath. After the chloroform has been completely evaporated, add 2 cc. of concentrated nitric acid, place a watch glass over the beaker, and boil until the gases evolved are colorless. Dilute to about 100 cc., heat to 75–80° C., add 2 cc. of potassium dichromate solution, and electrolyze, maintaining the stated temperature as directed in the preceding method. Dissolve the lead peroxide in a mixture of 2 cc. of potassium iodide, 2 g. dissolved in water and made up to 100 cc., and 4 cc. of acid sodium acetate solution, consisting of 20 cc. of saturated sodium acetate solution, 10 cc. of glacial acetic acid and 70 cc. of water. Titrate with approximately 0.001 *N* sodium thiosulfate solution that has been previously standardized against a known amount of lead as has been de-

[8] Cassil and Smith, *Am. J. Pub. Health* **26**, 902 (1936).

scribed in the foregoing. The very dilute thiosulfate solution may be protected from decomposition for several weeks by adding 1 per cent of amyl alcohol to the boiled water with which it is prepared.

If interferences are likely to be present, as for instance if canned goods are being examined and tin be possibly present, it is best to proceed with the Wichmann-Clifford method.

COPPER

Copper often occurs in foods that have been processed in copper kettles. The copper is oxidized to copper carbonate which is soluble in organic acids. Thus tomato products often are contaminated with copper. Copper salts are sometimes added to intensify the green color of chlorophyll. The views on copper contamination have undergone changes and it is now accepted that minute amounts of this metal are needed for proper human metabolism. In the methods detailed the copper is separated from the other metals by means of a sulfide precipitation and it is subsequently estimated by iodine liberated in the cupric-cuprous iodide reaction.

Weigh 100 g. of the sample into a porcelain dish, about 9–10 cm. and add 5 cc. of a mixture of 5 cc. of sulfuric acid and 95 cc. of 95 per cent alcohol. Burn to a white ash. Add 100 cc. of water after cooling and hydrochloric acid until slightly acid. Try to dissolve all of the ash. Filter and if necessary burn again. Pass in hydrogen sulfide for 15 to 20 minutes. Filter through quantitative paper. Wash. Place the filter paper plus the precipitate directly into a 100 cc. flask, a squat flask with a wide mouth, customarily called a "fat" flask, is preferable and add 3–4 cc. of sulfuric acid and 6 cc. of nitric acid. Place glass hooks on the flask and cover with a watch-glass. If the resultant mixture is dark add nitric acid until it is clear. Evaporate to 1 or 2 cc., cool, add 30 cc. of water and an excess of bromine water. Place the flask on the steam bath until the solution is colorless, after which cool and add ammonium hydroxide. In case iron is present, filter and then evaporate off the ammonium hydroxide. Make acid with acetic acid and titrate with 0.01 N thiosulfate in the presence of about 5 g. potassium iodide and starch solution. Factor on 0.01 N thiosulfate solution divided by 2 multiplied by the titration equals the mg. of copper per 100 g.

The A. O. A. C. gives the following alternative method. Dissolve the ash in hydrochloric acid, neutralize with ammonium hydroxide, add 5 cc. of sulfuric acid, dilute to 200 cc. and boil for 1 minute. Add cautiously

10 cc. hot, saturated solution of sodium thiosulfate and continue boiling for 5 minutes. Filter the precipitate, wash 6 times with hot water and reserve the filtrate for the zinc determination. Fold the filter paper, place in a crucible, and ignite in a muffle at 500° C. Treat the residue with 1 cc. nitric acid (2:5) and dry on a steam bath. Add 5 cc. of water and evaporate to dryness on steam bath. Add 20 cc. of water and an excess of ammonium hydroxide and heat until the copper salts dissolve. Transfer to a 100 cc. flask. Make acid to litmus with acetic acid (1:1) and add 1 cc. in excess. Boil for 1 minute and cool to room temperature. Add 2 g. of potassium iodide, dissolved in enough water to make the final solution 50 cc., and titrate the free iodine with 0.01 or 0.005 *N* sodium thiosulfate solution until the end point is nearly reached. Add 2 cc. of 1 per cent starch solution and continue titrating until the color is discharged.

Very small amounts of copper may be determined conveniently by the potassium ethyl xanthate colorimetric method. The copper, separated from other metals as the sulfide, is dissolved in nitric acid and a 0.1 per cent solution of potassium ethyl xanthate is added. The color developed is compared against standards treated the same way.

Zinc

Zinc salts are sometimes used instead of copper salts to increase the green color of vegetables. The metal sometimes contaminates powdered eggs that have been dried in galvanized iron containers, because the eggs are scraped from the container and some zinc gets scraped into the food material. If acid foods are processed in galvanized containers or pass through zinc lined vats or pipes, the food is likely to pick up some of the metal. In these procedures the zinc is separated from other metals by first precipitating the metals having sulfides, insoluble in relatively high acid concentration, the zinc remaining in the filtrate, and then estimating the zinc by precipitating it as zinc sulfide in a buffered faintly acid solution, thus separating the metal from those other metals whose sulfides are soluble in faintly acid solution.

Boil the filtrate from the copper determination to expel the hydrogen sulfide and reduce the volume to 250 cc. Add a drop of methyl orange indicator, 5 g. of ammonium chloride and make alkaline with ammonium hydroxide. Add hydrochloric acid (1:9) dropwise to faintly acid reaction, add 10–15 cc. of sodium or ammonium acetate, 50 g. of salt made up to 100 cc. with water, and pass in hydrogen sulfide until precipitation

is complete. Allow the precipitate to settle, filter, and wash twice with hydrogen sulfide water. Dissolve the precipitate on the filter with a little hydrochloric acid (1:3), wash the filter with water, boil the filtrate and washings to expel hydrogen sulfide and cool. Add a distinct excess of bromine water. Add 5 g. of ammonium chloride and ammonium hydroxide until the bromine disappears. Add hydrochloric acid (1:3) dropwise until the bromine color just reappears. Then add 10–15 cc. of sodium or ammonium acetate solution and 0.5 cc. of ferric chloride solution, 10 g. $FeCl_3.6H_2O$ in 100 cc. water, or enough to precipitate the phosphates. Boil until all the iron is precipitated. Filter while hot and wash the precipitate with water containing a little sodium acetate. Pass hydrogen sulfide into the combined filtrate and washings until all the zinc sulfide, which should be pure white, is precipitated. Filter through a weighed, prepared Gooch crucible and wash with hydrogen sulfide-ammonium nitrate water. Dry the crucible, ignite at a bright red heat, cool and weigh as zinc oxide, ZnO. Calculate the weight of metallic zinc, using the factor, 0.8034.

Zinc sulfide sometimes forms colloidal precipitates which will not flocculate and pass through the filter. Caldwell and Moyer[9] recommend the addition of a solution containing 0.5 to 2 mg. of gelatin of very low ash content. The gelatin solution will produce instantaneous and complete flocculation of as much as 0.3 g. zinc sulfide in 300 cc. of solution.

Tin

Canned food products sometimes contain tin, due to the action of fruit or organic acids, which exert a solvent action on the tin. It is best to obtain the tin for quantitative determination by the wet ash process, for heat ashing often yields low results. The tin is precipitated as stannous sulfide and separated from the sulfides insoluble in polysulfide by solution in polysulfide and filtration. The metal is then reprecipitated as the sulfide, and estimated as the oxide after roasting.

Add 200 cc. of water to the digested sample and transfer to a 600 cc. beaker. Rinse the Kjeldahl flask with 3 portions of boiling water, making a total volume of approximately 400 cc. Cool, and add ammonium hydroxide until just alkaline, then 5 cc. of hydrochloric acid or 5 cc. of sulfuric acid (1:3) for each 100 cc. of solution. Place the beaker, covered, on a hot plate. Heat to about 95° C. and pass in a slow stream

[9] Caldwell and Moyer, *J. Am. Chem. Soc.* 57, 2372 (1935).

of hydrogen sulfide for an hour. Digest at 95° C. for an hour and allow to stand 30 minutes longer. Filter, and wash the precipitate of stannous sulfide alternately with 3 portions each of wash solution and hot water. The wash solution consists of 100 cc. of saturated ammonium acetate solution, 50 cc. of glacial acetic acid, and 850 cc. of water. Transfer the filter and precipitate to a 50 cc. beaker, add 10–20 cc. of ammonium polysulfide, heat to boiling, and filter. Repeat the digestion with ammonium polysulfide and the filtration twice, and then wash the filter with hot water. Acidify the combined filtrate and washings with acetic acid (1:9), digest on a hot plate for an hour, allow to stand overnight, and filter through a double 11 cm. filter. Wash alternately with two portions each of the wash solution and hot water and dry thoroughly in a weighed porcelain crucible. Ignite over a Bunsen flame, very gently at first to burn off filter paper and to convert the sulfide to oxide, then partly cover the crucible and heat strongly over a large Meker burner. Weigh as stannic oxide, SnO_2 and calculate to metallic tin, using the factor, 0.7877.

Tin may also be determined colorimetrically by dissolving the purified stannous sulfide in 2.5 cc. of hydrochloric acid. Place this solution in a test tube fitted with a cork and delivery tube. Add a small piece of zinc and when it is dissolved, pass in carbon dioxide to replace the air, add 2 cc. of 0.2 per cent dinitrodiphenylaminesulfoxide in 0.1 *N* sodium hydroxide solution. Boil the mixture for a few minutes and dilute to 100 cc. Add a few drops of ferric chloride solution. The violet color so obtained may be matched against standard solutions of tin treated the same way.

Mercury

This metal generally comes in contact with food products because it is a component of some insecticides and fungicides. It is also the metallic constituent of many organic antiseptics and coloring matters, as for instance, merthiolate and mercurochrome.

Mercury may be detected directly in foodstuffs by applying the Reinsch test. This is carried out in a manner similar to the test for arsenic. A piece of pure burnished copper is immersed in a mixture of the material to be analyzed, with 1/5 its volume of hydrochloric acid, and the mixture is allowed to simmer for a number of hours. A bright lustrous mirror is formed on the copper in the presence of mercury. The piece of foil is washed with water, alcohol, and ether, dried, placed in

a subliming tube and heated. The mercury will deposit in the cool part of the tube, and may be identified under the microscope.

There are new dithizone methods for mercury. If large quantities are present, there is no particular advantage in these methods over that of the customary thiocyanate and sulfide methods. If small quantities are present, the mercury may be electrolytically determined, either in a nitric acid electrolyte, or using an alkaline sulfide electrolyte. The mercury may be deposited on a gauze cathode while employing rotating anodes with 2.5 amperes of current. The cathode is washed with water and dried in a desiccator. Do not dry mercury deposits in an oven. The cathode may be placed in a subliming tube and then heated. The mercury will sublime and deposit in the cool part of the tube.

Cadmium

Cadmium may occur in food products that have been produced or processed in cadmium plated vessels or molds. It is considered poisonous in small amounts. Cadmium may be detected in the wet ash solution after the total expulsion of nitric acid by precipitation as the sulfide, by separation from copper, if necessary, and reprecipitation as the sulfide.

Neutralize the wet ash solution, make slightly acid, but sufficiently so to hold all the zinc in solution, and pass in hydrogen sulfide. Yellow cadmium sulfide is precipitated. If copper is present, separate the cadmium from the copper. Redissolve the sulfides in either sulfuric or hydrochloric acid. Add an excess of sulfurous acid to make certain that no oxidizing substances are present and then add N ammonium thiocyanate solution. Copper precipitates as the dimeric cuprous thiocyanate, $Cu_2(SCN)_2$. Filter, and wash with cold water. Precipitate the cadmium in the filtrate and washings with hydrogen sulfide and estimate, in a manner similar to lead or antimony, by comparing with standard cadmium sulfide precipitates in Nessler tubes. This method has a large error since cadmium sulfide is often contaminated with a basic salt in the hydrogen sulfide precipitation.

Scott and Adams[10] recommend 1-(2 quinolyl)-4 allylthio-semicarbazide as a precipitant for cadmium in the presence of copper. One cc. of a 50 per cent ethyl alcohol saturated solution of 1-(2 quinolyl)-4-allylthiosemicarbazide, $C_3H_5NHCSNHNHC_9H_6N$, to 10 cc. of cadmium solution, gives a precipitate with 1 ppm. of cadmium in the presence of

[10] Scott and Adams, *J. Am. Chem. Soc.* **57**, 2541 (1935).

potassium iodide. Zinc, nickel, cobalt, sulfate and ammonia interfere. The metals may be eliminated by the usual sulfide separation, the sulfate with barium and the ammonia by evaporation.

Aluminium

Aluminium is not considered a toxic metal. However, it sometimes occurs in foodstuffs in fairly high amounts due to the addition of alum to pickling solutions and as a component of some baking powders. It may be determined simply with a fair degree of accuracy, in the presence of iron, by the Chancel method. The food material is ashed in the dry way and the silica dehydrated as usual. If no heavy metals are present, the residue is now taken up in 5 cc. of hydrochloric acid and 50 cc. of water, filtered and washed. The filtrate and washings are made up to about 200 cc. and dilute ammonium hydroxide is added until the precipitate which forms dissolves with difficulty. An excess of sodium thiosulfate is added and the solution boiled carefully, until all of the sulfur dioxide is expelled. Filter the precipitate of aluminium hydroxide on ashless filter paper and wash with 2 per cent ammonium nitrate solution. Ignite and blast in a tared crucible to constant weight. Cool and weigh as aluminium oxide, Al_2O_3. The reaction is:

$$2AlCl_3 + 3Na_2S_2O_3 + 3H_2O \rightarrow 2Al(OH)_3 + 6NaCl + 3SO_2 + 3S$$

The method, as may be seen, depends on the precipitation of aluminium as the hydroxide by boiling with sodium thiosulfate in neutral solution.

Small amounts of aluminium may be estimated colorimetrically as its lake with aurintricarboxylic acid which is also known as aluminon.

Nickel

Nickel is used as a catalyst in the hydrogenation of oils to make fats. It may at times contaminate the fats so produced. A simple and convenient means of detection and estimation in fats, is to isolate the nickel by solution in acid and subsequently, precipitate it with dimethylglyoxime or α benzildioxime. Place a weighed quantity of fat, about 250 g., in a flask, add an equal amount of hydrochloric acid and heat on a water bath for 1 to 2 hours with frequent swirling. Transfer the mixture to a separatory funnel and draw off the acid layer. Filter and evaporate in a porcelain dish on a water bath.

Dissolve the residue in 50 cc. of hot absolute alcohol, rendered just alkaline with ammonium hydroxide, and add 50 cc. of a hot saturated solution of dimethylglyoxime or α benzildioxime. The mixture is heated for a few minutes on the bath and is then filtered through a tared Gooch crucible, washed with hot alcohol, dried at 110° C., cooled and weighed.

If α benzildioxime was used then the weight of precipitate multiplied by the factor, 0.1093 equals the weight of nickel. If dimethylglyoxime was used then the weight of precipitate multiplied by the factor, 0.2032 equals the weight of nickel.

Thallium

Thallium may sometimes contaminate food materials through use as an insecticide. It is best detected spectroscopically, thallium giving a green color to the flame. If sufficient metal is present in the sample, it may be precipitated as the chloride, thallous chloride, $TlCl$. This salt is soluble in hot water and may be separated from lead chloride, also soluble in hot water by means of sulfuric acid. It may be confirmed by precipitation in neutral solution of thallous chromate, Tl_2CrO_4, yellow soluble in nitric or sulfuric acid.

Small amounts of thallium may be estimated by a method similar to the cupric-cuprous iodide method by the liberation of iodine from thallic chloride, $TlCl_3$, by the addition of potassium iodide, with the formation of thallous iodide, TlI, and free iodine which is subsequently titrated by standard sodium thiosulfate solution. Copper, of course, interferes.

REFERENCES

Scott, "Standard Methods of Chemical Analysis," Van Nostrand, 4th ed. (1925).
Treadwell and Hall, "Analytical Chemistry," Wiley (1924).
Methods of the A. O. A. C. (1935).
Methods for the Determination of Lead in Foods, Wichmann, Murray, Harris, Clifford, Loughrey and Vorhes, J. Assoc. Official Agr. Chem., 17, 108 (1934).
Curtman, "Qualitative Chemical Analysis," Macmillan (1931).
McAlpine-Soule, "Prescott and Johnson's Qualitative Analysis," Van Nostrand (1933).
Deposition Manual, Wilkens-Anderson Co. (1936).

CHAPTER VI

MILK AND CREAM

MILK and cream, alone or in combination with other foods, comprise about one-sixth of the weight of food eaten by an average American family. Still the United States does not consume as much milk as some European countries do, on a per capita basis.

Milk is the whole, fresh lacteal secretion obtained by the complete milking of one or more healthy cows, excluding that obtained within 15 days before and 5 days after calving, or such longer period as may be necessary to render the milk practically colostrum free. The name, milk, unqualified, means cow's milk. It consists largely of water, milk fat, lactose or milk sugar, protein and mineral matter. These are probably present in some form of combination such as fat-protein, protein-mineral matter. The three main characteristic constituents are milk or butter fat, casein and lactose.

Milk is one of the most important foods in the human diet because it has many components present in very small quantities that are essential to growth and well-being. That this is true, follows from the fact that mammalian animals can live and thrive for weeks and months without the addition of other foods. There are other foods which contain these materials also but are not as easily assimilable as milk. Some of the minor constituents, but very important, are lactalbumin, lactglobulin, lactic acid, sodium, potassium, calcium, magnesium, chlorides, phosphates, citrates, iodine, cholesterol, lecithin, enzymes, and the vitamins A, B_1, B_2 and C.

COMPOSITION

Davies[1] gives as the proximate analysis of milk, based on the analysis of thousands of samples by various investigators of United States, England, Germany and Scotland:

Fat	3.71%
Solids not fat	8.99%
Total Solids	12.7%

The maximum, minimum and average percentage composition of the more important constituents of milk as compiled by Davies[1] from the work of many investigators and that of the New York Agricultural Experimental Station at Geneva are given in the following table:

TABLE 4. COMPOSITION OF MILK

	Maximum	Minimum	Average	N. Y. State
Water	90.0	82.0	87.3	87.1
Fat	7.8	2.3	3.67	3.9
Casein	....	1.5	2.86	2.5
Albumin	....	0.5	0.56	0.7
Total Protein	4.5	2.0	3.42	....
Lactose	6.0	3.5	4.78	5.1
Ash	0.9	0.6	0.73	0.7
Total Solids	18.0	10.0	12.69	12.9

A more complete description of the composition of milk can be obtained from Table 5, as compiled by Babcock.

Oleic, followed closely by palmitic, is the predominant fatty acid. Myristic, stearic and butyric follow in order. The remainder may be considered the minor fatty constituents.[2] Casein, albumin and globulin constitute about 93 per cent of the nitrogeneous constituents of milk, and the remainder, which includes amino acids and amides, phosphatides, purine substances, ammonia, choline, thiocyanate, and lactoflavine are to be regarded as the minor nitrogeneous constituents.[3]

The composition of milk varies with the breed of cow, as illustrated in Table 6, the time of year, the time of day, the portions of any one milking, the individuality of the cow, the age of the cow, the period of lactation, feeding, and other factors. Jersey, Guernsey, and Ayrshire give milk richer in fat than Holstein and Dutch Belt. In the fall and early winter richer milk is obtained than in the spring and early summer.

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1 Davies, "Chemistry of Milk," Van Nostrand (1936).

2 Hilditch, *Analyst* 62, 250 (1937).

3 Bushill, Lampitt and Filmer. *Analyst* 62, 260 (1937).

The evening milking yields richer milk than the morning milking. The first portions or "fore" milk of milk drawn in the milking process are poorer than the last portions or "strippings." The reader is referred to texts on dairying for complete information.

TABLE 5. COMPOSITION OF MILK

			butyrin caproin caprylin (trace) caprinin (trace)		glycerides of soluble and volatile acids.	0.3			
	butter fat	3.6					fat	3.6	
			butin (trace) myristin palmitin stearin olein		glycerides of insoluble and non-volatile acids	3.3			
milk 100.0			casein	3.00					total solids 12.7
			albumin lactoglobulin	0.60	containing nitrogen	3.8			
			galactin	0.20					
			fibrin (trace)						
	milk serum	96.4	milk sugar			4.5			
			citric acid			0.1			
			potassium oxide	0.175			solids not fat	9.1	
			sodium oxide	0.070					
			calcium oxide	0.140					
			magnesium oxide	0.017					
			iron oxide	0.001					
			sulfur trioxide	0.027	ash	0.7			
			phosphorus pentoxide	0.170					
			chlorine	0.100					
			water						87.3
									100.0

The food analyst is, however, little concerned, except theoretically and from the investigational viewpoint, with these variations except with that of breed of cow, for his main interest lies in market milk, in which most of the variations disappear into an average due to mixing of herd milk on a large scale. The breed of cow has a large effect, as can be seen from Table 6. Thus for example, in the New York City milk shed, that is, the area surrounding New York City which supplies it with milk, probably 70 per cent of the cows are Holstein. These give milk of low fat content, circa 3.35 per cent whereas the usual average is much nearer 3.7. Another factor which influences the fat content of market milk is the fact that milk is sold by the producer or farmer to the distributor on

the basis of the per cent fat. In order to get a better price for his milk, the farmer may siphon off the upper portions of the milk for sale and use the lower portion, say one-tenth, for other purposes. This obviously tends to increase the fat content and thus yield the farmer a better price.

TABLE 6. VARIATION IN COMPOSITION OF MILK DUE TO BREED

	Fat	Lactose	Protein	Ash	Water
Jersey........	5.43	4.85	3.96	0.75	85.01
Guernsey.....	5.16	4.80	3.92	0.75	85.37
Ayrshire......	4.09	4.57	3.27	0.69	87.38
Shorthorns...	3.91	4.80	3.27	0.73	87.29
British Friesians...	3.63	4.62	3.11	0.71	87.93
Dutch Belt...	3.60	5.00	2.62	0.68	87.97
Holstein......	3.39	4.89	2.99	0.69	88.04

Due consideration must be given to the fact that much of the data on milk in the literature are results obtained from few samples. Thus it is well known, that although the minimum standard for milk fat percentage in many states of the United States and also in England is 3 per cent, milk obtained from some herds actually falls below that figure. Hence the minimum selected is not one which actually occurs but is one which has been adopted as a health measure.

Some other significant factors concerning milk are the following. The yield of milk is inversely proportional to the energy value, that is the calorific value, of the total solids per unit weight of milk. In other words, the greater the total volume of milk given by any one cow, the lower the total solid content.

Milk possesses the same osmotic pressure as blood and since the osmotic pressure of blood is and must remain practically constant, it follows that the osmotic pressure of milk should also be practically constant. The osmotic pressure is dependent on the number of dissolved particles in a solvent. The freezing point also depends on the number of particles

dissolved in a solvent and there is a mathematical relationship between these factors and other colligative properties such as the vapor pressure and the boiling point. Consequently the freezing point of milk also varies slightly and may be considered an index of a normal milk. In Chapter II, "Physical Chemical Methods," freezing point depression determination is outlined.

Specific Gravity

Milk is a fat-water emulsion, consequently its specific gravity is a function of the specific gravity of the fat and of that of the water solution. The specific gravity of the fat is about 0.93 and that of the solids not fat is 1.5. Hence, as the fat content of the milk increases, the specific gravity decreases and conversely, as the solids not fat content increases, the specific gravity of the milk also increases. The actual specific gravity found is some function of the two. The specific gravity of milk is usually obtained by means of a lactometer although it may also be obtained with a pyknometer. The New York Board of Health lactometer is a hydrometer graduated with arbitrary scale divisions from 80° to 120°, in which 100° equals 1.029, the average specific gravity of milk. This lactometer is calibrated at 60° F. and should be read at that temperature otherwise corrections need be made, by adding 2° of the arbitrary scale for every 5° F. rise and subtracting 2° of the arbitrary scale for every 5° F. below 60° F.

Another type of lactometer is the Quevenne with a scale divided into 25 equal parts from 15 to 40 in which 29 equals 1.029, the average specific gravity of milk. This instrument is also calibrated at 60° F. and should be read at that temperature. It may be corrected for readings not made at this temperature by adding 0.1 to the reading for each degree F. above 60° F., or subtracting 0.1 for each degree F. below 60° F.

The milk is stirred by passing from vessel to vessel, taking care to occlude as little air as possible, and is placed in a suitable cylinder. The lactometer is immersed in the fluid and is allowed to rise to its proper level. This is done in order to overcome surface tension and viscosity effects. The reading is made, the temperature taken, and the reading corrected for temperature. If a New York Board of Health lactometer is used the reading must be multiplied by 0.29 to convert to the Quevenne scale. The specific gravity may be obtained from the Quevenne reading by placing 1.0 before that scale reading, in other words, the readings form the hundredths and thousandths place after 1.0.

Fat

There are many methods for the determination of fat in milk. Of these, the Babcock and Gerber methods are the quickest and simplest. The Roese-Gottlieb is the best of the longer and more accurate methods.

Babcock Method—This method depends on the solution of all constituents of milk except fat and lipoid bodies in sulfuric acid, and the subsequent estimation of the fat by centrifuging into a graduated narrow neck of a special flask as the supernatant layer over the heavier layer of sulfuric acid.

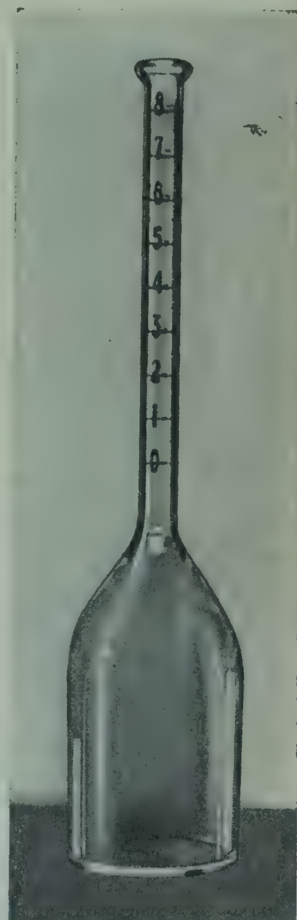

Fig. 39. Babcock Milk Flask

Measure 18 g. of milk from a properly mixed sample into a standard state branded milk test bottle; Fig. 39. by using a 17.6 cc. standard state branded pipette, add 17.5 cc. of standard commercial sulfuric acid, specific gravity 1.813, which is best and avoids charring the fat layer, and shake until all the curd has disappeared, then continue the shaking for about one-half minute longer. Before mixing, the milk and acid should have a temperature of about 60° F., if not, the amount of acid must be adjusted to give the proper rate of color development.

Place the test bottles in the Babcock centrifuge and whirl at the proper speed for five minutes, then fill the bottles with hot water, having a temperature of at least 200° F. to the bottom of the neck. Whirl for two minutes and fill with hot water at 200° F. to the top of the graduations and whirl again for one minute. If a large number of samples are to be read, place these samples in a water bath at 135° F. to 140° F. for five minutes. Read the per cent fat by measuring from the lowest point of the fat column to the highest point of the meniscus at the top of the fat column. Discard all results that do not have a clear fat column. Some centrifuge machines are equipped with heating apparatus. When using such machines, the temperature of the water added should be not less than 160° F. The

Babcock method makes use of standard pipettes and bottles whose specifications are rigidly drawn.[4]

Gerber Method—This method depends on the solution of all milk constituents in sulfuric acid other than fat using amyl alcohol to help

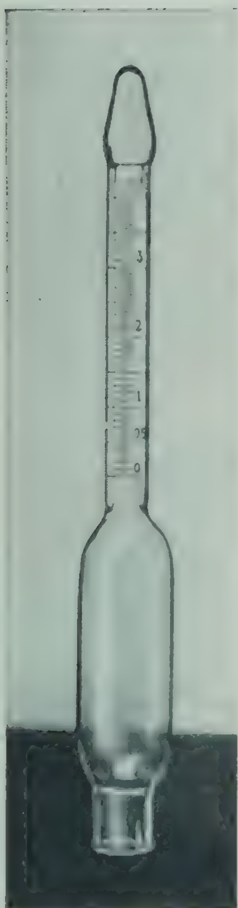

FIG. 40. Gerber Milk Butyrometer

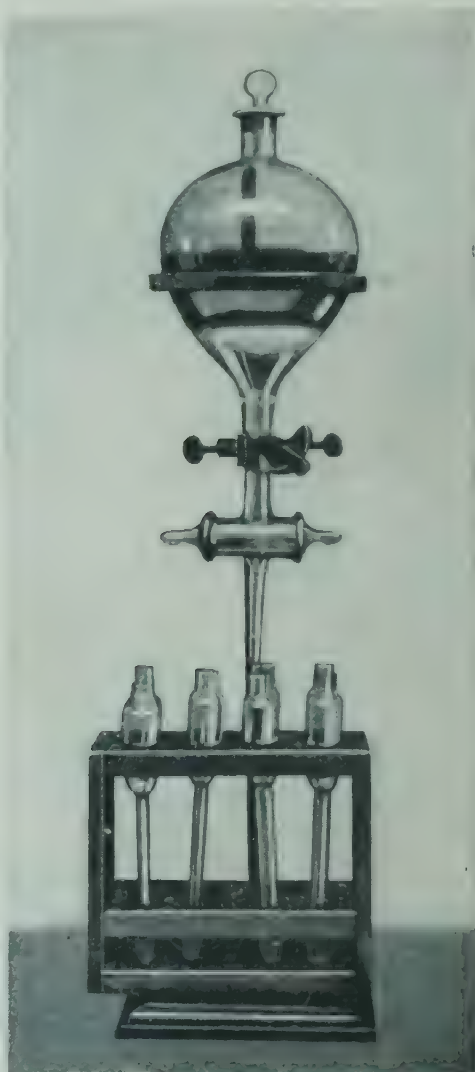

FIG. 41. 10 cc. Automatic Burette and Butyrometers

break the milk emulsion and prevent charring of the fat layer. The amyl alcohol should be pure and should be tested by running a control

[4] Dept. Agr. Markets, State of New York, Circ. No. 505 (1935).

exactly as detailed in the method, using water instead of milk. The fat reading should be zero. The amyl alcohol reacts with the sulfuric acid forming an ester which is completely soluble in the sulfuric acid and hence has no effect on the fat result. The fat is subsequently estimated by centrifuging, with the lipoids forming the supernatant layer in the capillary graduated portion of a butyrometer, Fig. 40. This method has the marked advantage that centrifuging only once is necessary. If the control test with the amyl alcohol gives an apparent fat reading, it must be rejected. Only amyl alcohol giving no fat reading may be used in this method.

Measure 10 cc. of sulfuric acid (sp. gr. 1.82) into the milk butyrometer, Fig. 41. Carefully add exactly 11 cc. of the milk sample equivalent to 11.33 g. with an 11 cc. pipette and add 1 cc. of amyl alcohol. The temperature of acid and milk should be near 60° F. When ready to mix, insert the stopper and shake. When the milk curd is completely dissolved, invert the bottle several times to mix the acid remaining in the neck of the bottle with the rest of the mixture. Place in a Gerber centrifuge or in adapters in a Babcock centrifuge and whirl for 5 minutes at the proper speed for the machine that is being used. The machine, if heated, should be at a temperature of about 160° F. A heated machine usually gives more satisfactory results. Remove and read immediately. By adjusting the stopper, the bottom of the fat column can be made to coincide with the zero or maximum division on the scale. The extreme lower part of the upper surface or meniscus of the fat column is read. When it is not possible to adjust the lower surface of the fat column to zero it may be adjusted to any other whole per cent mark and the proper calculation made when reading the test. The reading gives the per cent fat directly. The Gerber method makes use of standard pipettes and butyrometers whose specifications are rigidly drawn. This equipment is also known as state branded glassware.[5]

Roese-Gottlieb Method—The Roese-Gottlieb method replaces successfully the more tedious continuous extraction methods such as the Adam's coil method. It depends on the use of ammonia to soften the curd of the milk, of ethyl alcohol to break the milk emulsion and the fat-protein combination and of mixed ethers to extract the fat. The alcohol assists the ethyl ether in coming in contact with the fat. The petroleum ether is used to decrease the solubility of water and alcohol in the ethyl

[5] Dept. Agr. Markets, State of New York, Circ. No. 515 (1936).

ether and of course thus decrease the solubility of salts in the ether layer. The petroleum ether also decreases the solubility of ethyl ether in the water layer. The fat thus extracted is subsequently estimated by weighing. In performing these extractions, the Jacobs-Singer separatory flask or Mojonnier extraction tube are to be preferred as far superior to the unwieldy Röhrig tube or similar apparatus.

Transfer 10 g. of the milk sample to the lower section of a Jacobs-Singer separatory flask, Fig. 11, either by weighing directly into the flask, or by weighing by difference with the aid of a Mojonnier 10 g. pipette and carriage, Fig. 42, or by means of a calibrated delivery weight pipette. A Mojonnier extraction tube, Fig. 12, may also be used. Stopper the Jacobs-Singer separatory flask with the upper section. Add 1.25 cc. of

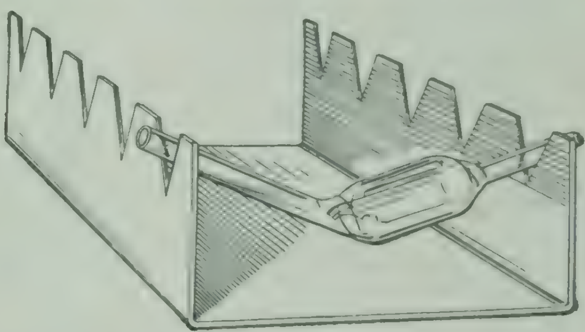

FIG. 42. Mojonnier Weight Pipette and Carriage
(Courtesy of Mojonnier Brothers)

ammonium hydroxide, or 2 cc. if the sample is sour, and mix thoroughly. Add 11 cc. of 95 per cent alcohol and mix well. Add 25 cc. of ethyl ether, shake vigorously for 30 seconds, add 25 cc. of petroleum ether and shake again for 30 seconds. Let stand for 20 minutes, or until the upper layer is perfectly clear, or centrifuge. Draw off as much as possible of the mixed ether layer into a tared "fat" flask, so designated because it is squat in form, through a small, quick-acting filter. Again extract the liquid remaining in the tube or flask, this time with 5 cc. of 95 per cent alcohol, and 15 cc. of each ether, shake vigorously 30 seconds after each addition and allow to settle. Draw off the clear solution through the small filter into the same tared flask. Repeat the extraction with another 15 cc. portion of each ether, shake vigorously 30 seconds after each addition and allow to settle. Draw off the clear solution through the small filter into the same tared flask. Add water to the separatory flask or to the extraction tube with the aid of a wash bottle until the level of the water layer reaches the middle of the constriction of the extraction

tube or the connecting joint of the separatory flask. Only a few ccs. will be necessary. Draw off the remaining ether solution through the small filter into the same tared flask as carefully as possible. Evaporate off the ether in the tared flask after each extraction slowly, on a steam bath, while the subsequent extraction is allowed to settle. Wipe off the outside of the flask and place in an oven thermostatically controlled at 100° to 105° C. Weigh the flask with a similar flask as a counterpoise after cooling in a desiccator. If a 10 g. calibrated weight pipette was used, the weight of the fat in the flask divided by 10 and multiplied by 100 equals the per cent fat in the milk sample.

Total Solids

Weigh into a tared flat-bottomed dish, containing 10 to 15 g. of pure dry sand, 5 g. of milk with the aid of a pipette or by difference. Place in an oven thermostatically controlled at 99° C. for four hours. Place into a desiccator and weigh quickly when cool; report the increase in weight as total solids.

Total Protein

Determine the nitrogen in 5 g. of the milk added to an 800 cc. Kjeldahl digestion flask by means of a weight pipette, as described in the Roese-Gottlieb method, by the Kjeldahl-Gunning-Arnold method detailed in Chapter I. Multiply the percentage of nitrogen by 6.38 to obtain the equivalent percentage of milk proteins.

Casein

The casein is separated from albumin and other proteins by precipitation with acetic acid and is subsequently estimated by determining the amount of nitrogen in that precipitate. To 10 g. of milk add 90 cc. of water at 40–42° C. and then 1.5 cc. of 10 per cent acetic acid. Stir and allow to stand 3–5 minutes. Decant on a filter, wash by decantation with cold water and transfer the precipitate to the filter. Wash twice on the filter. Determine the nitrogen in the washed precipitate and filter by the Kjeldahl-Gunning-Arnold method and multiply by 6.38 to obtain the percentage of casein. This determination must be made on the fresh sample. The milk should be preserved with formaldehyde, 1 part to 2,500 parts of milk, if the analysis is not made shortly after receipt.

Formol Titration of Casein The following method devised by McDowall and McDowell[6] is based on the fact that the addition of formaldehyde to a protein endows the protein with acidic properties. This method differs from the Walker formaldehyde-volumetric casein test in that the casein is separated from the milk by precipitation, and is redissolved in alkali before the addition of formaldehyde.

Twenty cc. of milk is diluted with 100 cc. of water at 42° C., but not higher in order to avoid coagulating soluble protein, in a 150 cc. beaker. Add at once 1.5 cc. of 1.67 *N* acetic acid (10 per cent), and then stir gently by rotating the stirring rod four times in the beaker. After allowing the beaker to stand about 20 minutes, add 4.5 cc. of 0.25 *N* sodium acetate solution and after stirring gently allow to stand for at least an hour.[7]

Decant the mixture through filter paper under gentle suction on a Büchner 6 cm. funnel. Wash the precipitate with water and allow to settle. Again decant the liquid through the filter paper. It is advisable to disconnect the suction pump as soon as all the liquid has passed through the filter, otherwise any casein that has passed over on to the filter will form an impervious layer. Repeat the washing and decantation a second time and finally transfer all of the precipitate to the funnel. Suction should cease well before the precipitate is dry. The precipitate and filter is returned to the original beaker. The funnel is inverted and washed with a little water to remove adhering particles. Add 4 to 5 cc. of 0.1 *N* sodium hydroxide solution. The total volume including the precipitate should now be about 20 cc. The beaker is placed in a boiling water bath for 5 minutes and is shaken occasionally until all the casein is dissolved and the fat emulsified. The milky solution is cooled to 21–24° C. One cc. of 1 per cent phenolphthalein is added and 0.1 *N* sodium hydroxide is added until an end point is reached matching that of 20 cc. of milk tinted with a few drops of 0.01 per cent aqueous rosaniline acetate solution. Use a portion of the sample of milk being analyzed, if possible, for the control. Add 4 cc. of 40 per cent formaldehyde of analytical quality and continue the titration with 0.1 *N* sodium hydroxide until the same end point is reached. The number of cc. of 0.1 *N* sodium hydroxide used in the second titration multiplied by the average factor 0.92 gives the percentage casein in the sample. The factor actually varies from 0.89 to 0.94, but the factor recommended gives results accurate to ±0.05 per cent.

[6] McDowall and McDowell, *Analyst* 61, 824 (1936).

[7] Moir, *Analyst* 56, 147 (1931).

Albumin

Exactly neutralize the filtrate obtained in the method for casein described in the section, "Casein," with 10 per cent sodium hydroxide solution, add 0.3 cc. of acetic acid (1 : 9), and heat on a steam bath until the albumin is completely precipitated. Collect the precipitate on a filter, wash with cold water, determine nitrogen as directed in the Kjeldahl-Gunning-Arnold method, and multiply by 6.38 to obtain the equivalent of albumin.

Minor Nitrogeneous Constituents [8]

The trichloroacetic acid precipitation will give the most satisfactory results for precipitation of the proteins. Ammonia may be estimated by steam distillation under reduced pressure. Urea may be determined by a modification of the urease method and creatinine may be estimated by a modification of the colorimetric method with picric acid, after the influence of lactose has been eliminated. Non-protein nitrogen may be determined on the trichloroacetic acid filtrate.

Ash

Add to a tared porcelain dish by means of a weight pipette 20 g. of the sample, add 6 cc. of nitric acid and evaporate to dryness. Ignite at a temperature below redness until the ash is free from carbon. Cool in a desiccator, weigh and report the increase in weight as ash.

Acidity

Place 17.6 cc. of milk by means of a Babcock pipette into a 125 cc. flask and dilute with an equal volume of water, recently boiled and cooled, washing out the pipette with this water. Titrate with 0.1 *N* sodium hydroxide solution, using 0.5 cc. of phenolphthalein indicator. The number of cc. of 0.1 *N* sodium hydroxide solution required divided by 20 gives the percentage of lactic acid.

Lactose

Dilute 25 g. of the sample with 400 cc. of water in a 500 cc. volumetric flask, add 10 cc. of copper sulfate solution, 34.639 g. of copper sulfate,

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[8] Bushill, Lampitt and Filmer, *Analyst* 62, 260 (1937).

$CuSO_4.5H_2O$, dissolved in water, diluted to 500 cc. and filtered through an asbestos mat, and 8.8 cc. of 0.5 *N* sodium hydroxide solution or an equivalent amount of potassium hydroxide solution. The alkali added should be sufficient to precipitate completely the copper as hydroxide from 1 volume of the copper sulfate solution. Fill the flask to the 500 cc. mark, mix, filter through a dry filter, and determine lactose in an aliquot by one of the copper reduction methods described in the chapter on sugars, Chapter IX.

Added Water

To 1 volume of copper sulfate solution, 72.5 g. of copper sulfate, $CuSO_4.5H_2O$, per liter, adjusted if necessary to read 36 at 20° C. on the scale of the immersion refractometer, or to a sp. gr. of 1.0443 at 20° C./4° C., add four volumes of milk. Shake well and filter. Determine the refractometer reading of the clear serum, that is the filtrate, at 20° C. A reading below 36 indicates added water.

Rapid Method

A fairly complete means of determining the chemical quality of milk is to combine the use of the lactometer for estimating the specific gravity and the Babcock method for estimating the per cent fat content. Then by the use of these two factors and a mathematical formula or table the total solids may be ascertained. The milk sample or samples are allowed to come to approximately 60° F., the lactometer readings are taken with a New York Board of Health lactometer, as described above, and recorded. The fat is then ascertained by the Babcock method. These two results may then be substituted in the formula of Hehner and Richmond [9] modified for the New York Board of Health lactometer readings to give the calculated total solids.

$$T.S. = \frac{L \times 0.29}{4} + 1.2F + 0.14$$

in which,

$T.S.$ = per cent total solids
L = New York Board of Health lactometer reading,
F = per cent fat

[9] Hehner and Richmond, *Analyst* 17, 170 (1892).

Let us assume that the lactometer reading was 107, corrected to 60° F. and that the fat content was 3.8 per cent, then by substitution

$$T.S. = \frac{107 \times 0.29}{4} + [1.2 \times 3.8] + 0.14$$

then,

$$T.S. = 12.46$$

Other formulas have been suggested by Babcock and other investigators. One of these that agrees closely with the total solids of milk of the New York City milk shed determined gravimetrically, and undoubtedly is applicable to herd milk in general, is the following:

$$T.S. = 1.2[F - 3.0] + 0.07[L - 100] + 10.89$$

in which,

$T.S.$ = per cent total solids
L = New York Board of Health lactometer reading at 60° F.
F = per cent fat.

Thus for the above example, we have by substitution,

$$T.S. = 1.2[3.8 - 3.0] + 0.07[107 - 100] + 10.89$$

then,

$$T.S. = 12.34$$

Gelatin and other Thickening Agents

A rare type of adulteration of milk is the addition of gelatin, or the addition of other thickening agents. These are much more likely to occur in cream or other milk products and will be discussed fully under those sections.

Preservatives

The detection and estimation of preservatives have been fully detailed in Chapter IV, "Preservatives in Foods." These methods may be applied successfully to milk.

Pasteurized Milk

Many cities and towns do not permit the sale of raw or improperly pasteurized milk because such milk may constitute a health hazard. The Food and Drug Administration, United States Department of Agriculture, defines pasteurized milk as milk every particle of which has been subjected to a temperature not lower than 142° F. for not less than 30 minutes and then promptly cooled to 50° F. or lower. In England and other countries, however, milk must be held for a half hour at 145° to 150° F. for proper pasteurization. In New York City, milk must be held at 143° F. for 30 minutes to be properly pasteurized. By improper pasteurization is meant, then, a milk which has not been held at 142° to 143° F. for the proper length of time, namely, 30 minutes, or one in which the temperature of pasteurization was less than 142° to 143° F.

The correct process of pasteurization entails expense and an unscrupulous distributor may improperly pasteurize milk, or may add raw milk to standardize, his product, that is, adjust the chemical composition by addition of milk, in order to lower costs. Many times milk is improperly pasteurized due to carelessness or poor equipment. At any rate, this is an important problem in the regulation and control of the sale of milk. This problem has been attacked from the fact that many, if not all of the enzymes in milk are heat labile at the temperature of pasteurization and because of the holding-time in the pasteurization process. This is the basis of many of the older as well as newer tests for heated and pasteurized milk.

Schardinger Test—The Schardinger[10] test is based on the destruction of the peroxidase enzyme in the pasteurization process. Mix 20 cc. of milk in a test tube with 1 cc. of a solution containing 5 cc. of a saturated alcoholic solution of methylene blue and 5 cc. of 40 per cent formaldehyde and 190 cc. of water. Cover the contents of the tube with a layer of liquid petroleum to prevent access of air and place the tube in the water bath at 45–50° C. Raw milk will decolorize this reagent in less than 20 minutes. Pasteurized milk will take longer than 20 minutes. This test is not satisfactory for detecting improperly pasteurized milk but is of historical interest and may be used as a rapid test for the distinction between raw and pasteurized milk.

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[10] Schardinger, *Z. Nahr. Genussm.* 5, 1113 (1902).

Leahy Test—The Leahy [11] test is based on the assumption that amylase is completely inactivated by heating milk at 143° F. for 30 minutes. Ten cc. of a well mixed sample of the milk is placed in a test tube by means of a pipette with precautions to prevent contamination with raw milk or salivary amylase. At a recorded time 0.5 cc. of a sodium chloride-starch reagent, prepared by dissolving 0.4 g. of soluble potato starch and 20 g. of salt in 100 cc. of water, is added and mixed well by pouring from one test tube to another and back again. The mixture is incubated at 30° C. for 4 hours. At the end of the incubation period, 2 cc. of a reagent consisting of equal volumes of glacial acetic acid and chloroform are added followed by immediate shaking with the thumb over the mouth of the tube. The addition of this strongly acid reagent, which changes the pH to about 3.4, terminates completely the activity of any milk amylase present. Thereafter, at a convenient time the tube is centrifuged at high speed for about 15 minutes. The clear supernatant liquid resulting from this treatment is poured into a test tube and the stage of starch hydrolysis effected by the enzyme determined by the addition of a few drops of dilute iodine solution such as 0.5 *N* iodine stock solution diluted to 0.005 *N* strength. This dilution should be prepared just before use.

The stage of starch hydrolysis indicated by the color developed on the addition of dilute iodine solution measures the temperature at which the milk was heated. Blue denotes pasteurized milk, yellow denotes 5 per cent or more of raw milk and intermediate colors of red, purple and orange denote improper pasteurization.

This test and also that of Gould [12] and the older one of Rothenfusser [13] do not, for unknown reasons, always distinguish between pasteurized and improperly pasteurized milk and sometimes do not even distinguish between pasteurized and raw milk. Hence, caution must be observed in interpreting results with these procedures.

Kay and Graham [14] Phosphatase Method—The inadequacy of all other tests in distinguishing between pasteurized and improperly pasteurized milk led to the development of the phosphatase test of Kay and Graham. This test is based on the distinction produced by the complete

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[11] Leahy, Intern. Assoc. Dairy Milk Inspectors, Ann. Rep. 23, 93 (1934).

[12] Gould, *J. Dairy Sci.* **15,** 230 (1932).

[13] Rothenfusser, *Z. Untersuch. Lebensm.* 60, 94 (1930).

[14] Kay and Graham, *J. Dairy Research* **6,** 191 (1935).

or incomplete destruction of the enzyme phosphatase by heating. Inorganic phosphate and phenol are liberated from a buffered solution of disodium-phenylphosphate by the action of phosphatase which is present in raw or improperly pasteurized milk, or in mixtures containing raw or improperly pasteurized milk. The phenol liberated is estimated by the use of the Folin and Ciocalteu reagent.

Reagents. A) Dissolve 11.54 g. of sodium barbital or sodium veronal and 1.09 g. disodium-phenylphosphate in water saturated with chloroform and make up to a liter with water saturated with chloroform. The disodium-phenylphosphate may sometimes be contaminated with phenol. It may be purified from any such contamination by washing with ethyl ether until the washings give no test for phenol with 2–6 dibromoquinonechloroimide.[15] To perform this test, add 10 cc. of water to the last of the ether washings and evaporate off the ether. Adjust the pH to 9.6 by the addition of ½ cc. borax buffer [15] and add a few drops of 2–6 dibromoquinonechloroimide solution, 100 mg. dissolved in 25 cc. of 95 per cent alcohol. No development of a blue color indicates absence of phenol. To prepare the borax buffer, dissolve 15 g. of anhydrous sodium tetraborate powder in 900 cc. of warm water. Add 3.27 g. sodium hydroxide in the form of a 20–40 per cent solution and make up to a liter. Five cc. of this buffer added to 100 cc. of water should produce a pH of 9.6.

B) Folin and Ciocalteu reagent. Dissolve 100 g. of sodium tungstate, $Na_2WO_4.2H_2O$ and 25 g. of sodium molybdate, $Na_2MoO_4.2H_2O$ in 700 cc. of water in a 1,500 cc. flask connected by ground glass joint to a reflux condenser. Add 50 cc. syrupy 85 per cent phosphoric acid and 100 cc. of hydrochloric acid. Reflux the mixture gently for 10 hours. After this time, cool, add 150 g. lithium sulfate, Li_2SO_4, 50 cc. water and 4–6 drops of liquid bromine. Boil the mixture under a hood without the condenser for 15 minutes to boil off excess bromine. Cool, dilute to 1 liter and filter. The finished reagent should have a golden yellow color with no greenish tint. Dilute with 2 volumes of water as needed for use.

C) A 14 per cent solution of anhydrous sodium carbonate.

Short Test. Ten cc. of solution A, the sodium veronal-disodium-phenylphosphate buffer, is placed in 4 test tubes of 25 cc. capacity. To two tubes, which act as controls, add 4.5 cc. of diluted Folin phenol reagent. To all 4 tubes add 0.5 cc. of milk and mix well. Incubate the two tubes without the Folin phenol reagent at 47° C. ± 2° in a water

[15] Stand. Meth. Water Analysis, Am. Pub. Health Assoc., 8th ed. (1936).

bath for 10 minutes. After this time remove from the bath, cool, add 4.5 cc. diluted Folin phenol reagent. Allow to stand for 3 minutes. Filter all four tubes. To 10 cc. of the filtrate, add 2 cc. of 14 per cent sodium carbonate solution. Mix, place in a boiling water bath for 15 minutes. Filter. Pasteurization or non-pasteurization is ascertained by the depth of the blue color produced.

Long Test. To 0.5 cc. of milk add 10 cc. of solution A, the sodium-veronal-disodium-phenylphosphate buffer plus 2 drops of chloroform and incubate for 24 hours at 37 to 38° C. To the above add 4.5 cc. of Folin phenol reagent, properly diluted. Let stand for at least 3 minutes and filter. Pipette 10 cc. into a test tube and add 2 cc. of 14 per cent sodium carbonate solution. Heat in a boiling water bath for at least 5 minutes and filter. Run in duplicate, and always run controls which are not incubated and to which diluted Folin reagent is added immediately. Compare the color produced with standards. A light blue indicates a pasteurized milk. A dark blue indicates an under pasteurized milk. A purplish color indicates a raw milk. Any color exceeding 2.3 Lovibond units of blue indicates milk not properly pasteurized. Gilcreas and Davis [16] recommend the production of blue colors by Folin's reagent with known phenol solutions as standards instead of Lovibond blue units. They found that a phenol value of 0.037 mg. per 0.5 cc. of sample examined or less indicates adequate pasteurization.

The Kay and Graham test was designed for milk pasteurized according to British standards, namely, at 145 to 150° F. for one-half hour. However, by suitable modification the phosphatase test can be adjusted to suit American standards. This is done by diminishing the length of time of incubation. If a given milk has been claimed to be heated for one-half hour at 142–143° F., it should not give more than 2.3 Lovibond blue units after 2½ hours incubation at 37° C. If it gives a greater color than this at this temperature of incubation then the milk is not properly pasteurized. On the other hand, the test may be made more sensitive for American pasteurization procedure by keeping the 24 hour incubation period and increasing the maximum allowable Lovibond blue units obtained to 4.

Modified Kay and Graham Method, Dye Reagents for Phenol.—The essential feature of the Kay and Graham method is the co-liberation

16 Gilcreas and Davis, Annual Proceedings Intern. Assoc. Milk Sanitarians, p. 15 (1936).

of phosphate and phenol. They estimate the phenol by means of the Folin and Ciocalteu reagent. Raybin [17] suggests the use of the Gibbs' reagent,[18] 2–6 dibromoquinonechloroimide and of p-dimethylphenylenediamine [19] as a means for detecting the liberated phenol directly within the milk. P-nitrosodimethylaniline [19] may also be used but must be reduced to the diamine by zinc dust prior to use. Other similar reagents may be used. The use of these reagents has advantages for rapid and not too sensitive work.

To 10 cc. of Kay and Graham reagent A, the sodium veronal-disodium-phenylphosphate buffer solution, add $\frac{1}{2}$ cc. of milk, mix stopper and maintain at about 37° C. for about 5 to 10 minutes. At the end of this period add 4 drops of a solution of 2–6 dibromoquinonechloroimide, 100 mg. of the dye intermediate reagent in 25 cc. of 95 per cent alcohol. Allow 5 to 10 minutes for the color to develop. A blue color, greyed over by the milk, indicates an improperly pasteurized milk. Properly pasteurized milk will remain unchanged or may develop a faint blue shade.

Preferably, the colors developed may be extracted by solvents. After allowing the color to develop, add 3 cc. of iso-butyl alcohol, amyl alcohol, or ethyl acetate and mix gently. Any blue color that was formed in the milk by the reaction between the phenol liberated by the phosphatase enzyme and the imide, with the formation of an indophenol, will be extracted by one of the aforementioned solvents. In iso-butyl alcohol and amyl alcohol, the color will remain blue, in ethyl acetate it will change to red. A deep blue or red color indicates a raw or improperly pasteurized milk. A properly pasteurized milk will not color the solvent layer, or will impart a mere tinge of red or blue, as the case may be.

The details of the test with p-dimethylphenylenediamine reagent are somewhat different. To 10 cc. of the Kay and Graham sodium veronal-disodium-phenylphosphate buffer add $\frac{1}{2}$ cc. of milk and allow to stand for 5 to 10 minutes at about 37° C. for about 5 to 10 minutes. At the end of this period add 4 drops of 5 per cent sodium bicarbonate solution, 5 g. sodium bicarbonate dissolved in water and made up to 100 cc., 4 drops of 0.1 per cent diamine reagent, 0.1 g. p-dimethylphenylenediamine dissolved in 100 cc. of water and then sodium hypochlorite solution containing 0.05 per cent of available chlorine until the pink color first produced changes to colorless or blue. The sodium hypochlorite solution may be

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[17] Raybin, personal communications (1936-7).

[18] Gibbs, *J. Biol. Chem.* 72, 649 (1927).

[19] Houghton and Pelly, *Analyst* 62, 117 (1937).

prepared by dissolving 60 g. of sodium carbonate and 40 g. of bleaching powder in 400 cc. of water, filtering and diluting the filtrate so that it contains 0.05 per cent of available chlorine. Allow the milk reaction mixture to stand for a few minutes for the color to develop and then shake out the dye formed with either chloroform or carbon tetrachloride. A blue color in the lower solvent layer indicates raw or improperly pasteurized milk or a mixture of the two.

There are no particular advantages in extending these tests to replace the more exact Kay and Graham procedure, although there are advantages in using them as rapid methods in plant control work. The tests should be performed with controls and may, as was previously stated, be performed without the addition of solvents and the subsequent extraction of the dyes formed by allowing the color to develop in the milk. In this case the blue color is greyed over by the milk, nevertheless, it is readily identified.

These rapid procedures have several limitations which would also inhere in any longer method using the same technique and reagents. It is well known that the organic reagents used in these methods are subject to decomposition. In the case of the 2–6 dibromoquinonechloroimide test, it must be understood that the indophenol formed, being an oxidation-reduction indicator, is subject to reduction to a colorless or leuco compound by a number of substances present in the milk, for example vitamin C. Hence, an excess of dye must first be formed over the amount of reducing material present before any blue color will develop, thus lowering the sensitivity of the test. Furthermore, minute amounts of other substances such as sulfides, sulfites, thiosulfates, hydrosulfites, thiourea, or possibly the sulfhydryl, glutathione, groups of the proteins of milk may change the color of the dye formed to red. Cysteine gives yellows with orange shades. Also, the development of color in the milk is subject to the correct pH, namely, 9.6 being maintained. For full development of color at least 4 hours standing or standing over night is recommended by "Standard Methods of Water Analysis." [20]

Homogenized Milk

The methods detailed above serve to give the composition of the milk analyzed. However, a milk could give almost normal constants for some and all factors and still be adulterated. The older ways of adulterating

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[20] Standard Methods of Water Analysis, Am. Pub. Health Assoc., 8th ed. (1936).

milk by skimming, watering and adding foreign materials have given place to much subtler methods. In the face of milk surpluses at one time of the year and shortages at other times, the reconstitution of milk is an ever provocative form of sophistication. In the following sections methods for the detection of such adulterations and sophistications will be discussed.

Milk may be so reconstituted that every one of the constants usually determined will be normal. In general, at least part or all of such milk will be homogenized. In some cities it is permissible to sell homogenized milk because it is assumed that such milk may be more easily digested. Another reason is that homogenized milk may be served from mechanical dispensers and still retain uniform composition. This permission is open to question because of the possibility of abuse.

Formerly it was considered sufficient evidence of homogenization to note the regularity of particle size under the microscope due to the belief that the colloid mill or other homogenizing machine produced an emulsion in which the particles are of equal size. This method has proved unsuccessful. Lampitt and Bogod [21] measured the degree of homogenization by estimating the residual fat in definite strata of milk after centrifuging at 2000 R. P. M. for 2 minutes.

Cream Line Method—This method is based on the observation that a completely homogenized milk will show no cream line on centrifuging at moderate speeds. Twenty cc. of milk are placed in a test tube, 15 × 150 mm., and whirled in a centrifuge at a speed near 1200 R. P. M. for a period of 2 minutes. The cream line produced is measured with a millimeter scale and is averaged. An unadulterated pasteurized milk gives an average cream line of 10 mm. with a minimum of 8 mm. A fully homogenized milk gives a 0 to 1 mm. cream line.

Curd Tension—This test measures the resistance of a knife to passage through a curd produced by the action of pepsin on milk. There are many processes for altering the properties of curd in milk in order to make the milk more digestible. One of the methods for measuring whether or not a milk has been treated to alter its curd is the Hill test modified by Otting and Quilligan.[22]

Six-tenths gram of scale pepsin, 1 to 3,000 (This ratio implies the

[21] Lampitt and Bogod, Chemie et Industrie Spe. No. 1004-09, Apr. (1934).

[22] Otting and Quilligan, Milk Dealer, Aug., p. 36 (1934).

pepsin is of such strength that it will digest not less than 3,000 times its own weight of coagulated and disintegrated egg albumin.], and 3.5 cc. of 0.1 *N* hydrochloric acid are made up to 100 cc. with water. This pepsin solution has a pH of 1.66 determined electrometrically. Ten cc. of the pepsin solution is placed in a suitable jar, brought to a temperature of 95° F. by placing in a water bath. One hundred cc. of the milk is brought to the same temperature and is added to the jar containing the pepsin and the Hill curd knife. The jar and contents are rotated for an instant and placed back in the water bath and held at this temperature for 10 minutes, after which the curd tension is determined.

Most milk has a curd tension varying from 60 to 250 grams. That is, there is a resistance equivalent to that weight in pulling the Hill curd knife through the curd. A soft curd milk, often a homogenized milk, will have a curd tension of zero to 30 grams.

Reconstituted Milk

The tests detailed in the preceding section merely distinguish between homogenized and non-homogenized milk and do not necessarily imply reconstitution. Tests for reconstituted milk depend on the estimation of components and properties that are heat labile or are likely to change due to the manner of processing or due to age. Some of the factors and properties that change readily are taste, odor, flavor, general appearance, vitamin content, ammonia content, sulfhydryl content,[23] fluorescent properties[24] and others. If for example, butter has been used to reconstitute milk and the butter was colored, the milk will contain color and would be obviously adulterated. If milk powder, either whole or skimmed is used and the milk is not properly homogenized, the sediment will generally be greater and the milk will not filter easily through a Fisher cotton filter pad placed in a Gooch crucible, or some similar arrangement.

If all of the gross characteristics of a milk are apparently normal, which will indeed be difficult in the matter of taste and odor in a fully reconstituted milk, reconstitution may be determined by the estimation of a heat labile component such as vitamin C or by ascertaining the quantity of ammonia which increases with age. Butter and most skim milk powders have little vitamin C because most of the vitamin is lost

[23] Beck and Urack, *Z. Untersuch. Lebensm.* **45**, 399 (1933).

[24] Radley, *Analyst* **58**, 527 (1933).

in the various processes for the production of the material. Vitamin C, or rather the iodine reducing materials of milk, may be estimated by the following procedure.

To 100 cc. of milk in a small beaker, add 8 cc. of 40 per cent trichloroacetic acid. Stir thoroughly, allow to stand 5 minutes and transfer to a centrifuge bottle. Centrifuge at 1400 R. P. M. for 5 minutes. Filter the supernatant liquid through a medium fast retentive filter paper. Refilter. Transfer 50 cc. of the filtrate to a small flask, add 1 to 2 cc. of starch solution and titrate with 0.01 *N* iodine solution which has been carefully standardized, to a blue color.

The above method is purely empirical and does not really give only the vitamin C content for all substances easily reduced are included. Nevertheless, the result is indicative of the quality of the milk tested. This test should not be performed on old milk, otherwise the results have little meaning. To illustrate the differences in reducing power of various milks the average vitamin C content of approximately two hundred samples are given:

raw milk	0.028 mg. per cc.
pasteurized milk	0.023 mg. per cc.
reconstituted milk	0.009 mg. per cc.

These averages are based on the assumption that 1 cc. of 0.01 *N* iodine solution is equivalent to 0.88 mg. of ascorbic acid. The lower and upper limits of vitamin content overlap in the case of raw and pasteurized milk but a milk containing 25 per cent reconstituted milk is suspicious and one containing 50 per cent is easily detected.

The Leahy, Kay and Graham, and dye modifications of the Kay and Graham tests cannot distinguish between a pasteurized milk containing 5 per cent raw milk and a full raw milk, nor indeed between a badly improperly pasteurized milk and a raw milk. In a measure, this makes all these tests inadequate. However, in conjunction with the iodine reduction test, a means of differentiating between a full raw milk and a milk which is improperly pasteurized or contains some raw milk is indicated. Thus for example, a high reduction and a very blue color would imply a full raw milk.

The quantity of ammonia increases markedly with age and quality of milk.[25, 26] The relationship is illustrated by the following data:

[25] Burstein and Frum, *Z. Untersuch. Lebensm.* 69, 421 (1935).
[26] Kluge, *Z. Untersuch. Lebensm.* 71, 232 (1936).

best quality	0.1 to 0.12 mg. per 100 cc.
medium quality	0.15 to 0.18 mg. per 100 cc.
poor quality	0.22 to 0.23 mg. per 100 cc.

Evenson[27] Method—This method for the detection of reconstituted milk is based on the production of a yellow color in the curd of remade milk. To 25 cc. of milk add 25 cc. of water and warm to 30° C. Add 4 cc. of 10 per cent acetic acid. Add 200 cc. of water and allow the curd to settle. Filter through bolting cloth or silk, wash 3 or 4 times with water and wash the curd back into the beaker. Wash 3 or 4 times by decantation, filter again and wash. Place the curd in test tubes or vials or in small beakers and add 10 cc. of 5 per cent sodium hydroxide solution. After 2 hours, a yellow color appears in the case of re-made milk. It is best to run a control on known milk along with the sample to be tested and compare the colors produced.

Foreign Fat

A milk containing foreign fat would obviously be adulterated. Foreign fat may be detected in a manner similar to that detailed in the section under cream.

Chloride Test

A test based on the fact that infected udder tissues allow the salts of the blood plasma to filter through into the freshly secreted milk was developed by Hammer and Bailey.[28] An increase over the normal amount of chloride in the freshly drawn milk therefore indicates an abnormal condition in the udder. In a similar respect, an increase in the chloride content of market milk indicates milk that comes from a poor source or milk that has been tampered with and possibly reconstituted. Thus the use of salt butter in reconstitution would very likely raise the chloride content far above normal. Place 10 cc. of milk in a flask and dilute with 40 cc. of water. Add 8 to 10 drops of 10 per cent potassium chromate solution, 10 g. K_2CrO_4 dissolved in water and diluted to 100 cc. The mixture is then titrated with 0.1 *N* silver nitrate solution. One cc. of 0.1 *N* silver nitrate solution is equivalent to 3.55 mg. chlorine.

A shorter method was suggested by Hayden.[29] Measure accurately 5 cc. of a silver nitrate solution, 1.3415 g. of silver nitrate dissolved in a

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[27] Evenson, *Intern. Assoc. Dairy Milk Inspectors, Ann. Rep.* 12, 354 (1923).

[28] Hammer and Bailey, Iowa State Coll. Agr. Mech. Arts, Bull. No. 41 (1917).

[29] Hayden, Cornell Veterinarian 22, 277 (1932).

liter of water, into a test tube. Add 2 drops of a 10 per cent potassium chromate solution and then exactly 1 cc. of milk. If the milk contains more than 0.14 per cent chlorides, a yellow color will develop in the tube, because all the silver has been precipitated as the chloride. This yellow color indicates abnormal milk for from general experience, it is assumed that the normal content of chlorides varies from 0.09 to 0.14 per cent. If the milk contains less than the stated amount of chloride the tube would have a color that varies from reddish to brownish depending upon the amount of silver chromate precipitated.

CREAM

Cream is that portion of milk, rich in milk fat, which rises to the surface of milk on standing or is separated from it by centrifugal force. In modern dairies, cream is almost invariably made by use of centrifugal separators. Cream is generally classified under three heads. Light cream containing 18 per cent fat, medium cream containing at least 25 per cent fat and heavy cream containing at least 36 per cent are the three designations. Since it is made from whole milk its three principle ingredients are milk fat, casein and lactose. Of these milk fat is the major ingredient of cream and most analyses are made with the object of ascertaining the percentage fat content.

Determination of Fat

The principles underlying the methods for estimation of fat in cream are similar to those discussed in the sections on milk.

Babcock Method—On cream-testing scales which are in proper working condition and of proper sensitiveness weigh nine grams, if a nine-gram bottle is to be used, Fig. 43, or eighteen grams if an eighteen-gram bottle is to be used, of the properly mixed sample into a standard cream test bottle. If nine grams of cream have been weighed, add 9 cc. of water at about 60° F. rinsing down the neck of the bottle with this water. If eighteen grams of cream have been weighed, no water need be added. Add 17.5 cc. of sulfuric acid, sp. gr. 1.82 at a temperature of 60° F.

After shaking, if the proper amount of acid of the correct strength has been used, the mixture of cream, water and acid or of cream and acid should be chocolate brown in color or the color of coffee to which cream has been added. After mixing the sample, whirl in a Babcock centrifuge at the proper speed for five, two, and one minutes, respectively.

filling the bottles with water at a temperature of about 200° F. to the bottom of the neck after the first whirling and to near the top graduation after the second whirling. The centrifuge machine, if heated, should be at a temperature of about 160° F.

If a large number of samples are to be read they should be placed in a tempering bath at 135–140° F., leaving them in the bath for 5 minutes before reading. Add a few drops of meniscus remover by allowing it to run down the inside wall of the bottle and spread over the fat. Read at once, preferably by using dividers. Read the cream test by measuring the fat from the lowest point of the fat column to the line which divides the fat column from the meniscus remover or leveler, observing all the precautions mentioned in the directions for estimating the percentage fat by the Babcock method in milk. Meniscus remover may be made by adding an oil soluble dye to Russian mineral oil until a deep red or blue color is obtained. The fat column may also be read without any aids as in the analysis of milk, but in this case from the lower surface to the bottom of the upper meniscus.

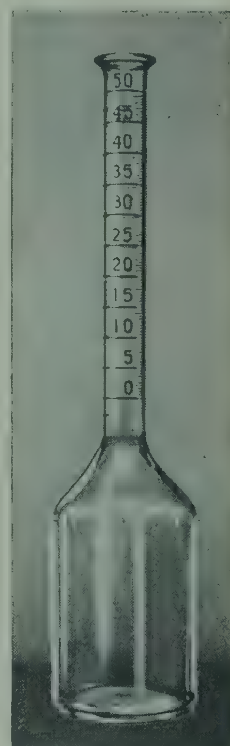

FIG. 43. Babcock Cream Flask

The fat column of all tests should be clear, translucent and have a golden yellow to amber color. All tests which are milky or foggy or show the presence of curd, charred matter or other foreign material in or below the fat column, or of which the reading is indistinct or uncertain should be rejected. Only test bottles that have the proper specifications should be used.[30]

Gerber Method—Measure 10 cc. of sulfuric acid, specific gravity 1.82, into the cream butyrometer, Fig. 44, and balance on a sensitive scale, properly protected against drafts. Weigh in, very carefully, 5 g. of the properly prepared cream sample, add 5 cc. of water and 1 cc. of amyl alcohol, insert the stopper and shake, inverting the bottle several times after the curd is all dissolved, as in the case of milk. Centrifuge for 5 minutes at the proper speed, remove and read the same as for milk. The fat column of the finished test should be clear, translucent and should

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[30] Dept. Agr. Markets, State of New York, Circ. No. 505 (1935).

have a golden yellow to amber color. All uncertain tests should be rejected. Only standard butyrometers [31] should be used.

Roese-Gottlieb Method—Transfer approximately 5 g. of cream accurately weighed, by weighing by difference with the aid of a Mojonnier pipette and a carriage to a Mojonnier extraction tube or a Jacobs-Singer separatory flask, or weigh directly into the lower section of the Jacobs-Singer separatory flask, about 5 grams of the sample, noting the exact weight. Add 5 to 6 cc. of water, according to the amount of sample being used, warm to 60° C. and then proceed as described in the method for milk. Care must be taken to add the full amount of alcohol. Divide the weight of fat found by the weight of the sample used and multiply by 100 to obtain the percentage fat.

Fig. 41. Cream Butyrometer

Determination of Other Constituents of Cream

Total solids, lactose, proteins and ash may be estimated as described previously under milk. Preservatives may be detected and estimated in a manner similar to those given in the chapter on preservatives, Chapter IV. Added coloring matters may be ascertained as detailed in the chapter on coloring matter in food, Chapter III.

Thickening Agents

Thickening agents are substances added to food and food products to increase the viscosity of those products. Closely allied to thickening agents are emulsifying and stabilizing substances. In ice cream and cheese manufacture, these agents form an important part of the production procedure. In other milk products, the use of these materials is of doubtful value. Gelatin, sucrate of lime, starch, gums and pectins are among the substances generally used.

Gelatin—Gelatin has for many years been used as a thickening agent in dairy products. In ice cream it has, of course, been accepted because

[31] Dept. Agr. Markets, State of New York, Circ. No. 515 (1936).

it improves the texture as well as the standing qualities of this product. However, in milk, cream and like products, its use has generally been frowned upon because of a tendency to use it to conceal inferiority. The methods of Stokes, Jacobs and Jaffe, and Richardson and Tarassuk are detailed. The method of Stokes is adequate for milk and sweet cream, that of Jacobs and Jaffe is adequate for sour milk products, generally. The Richardson and Tarassuk method is much longer than either of the others but is very valuable where doubtful results are obtained by the other methods.

Stokes Method.[32] The method mainly used for the detection of gelatin in dairy products has been that of Stokes. It depends on the precipitation of the milk proteins by acid mercuric nitrate and the subsequent detection of gelatin by the gelatin-picrate reaction. It is performed in the following manner: To 10 cc. of milk or cream or milk product, add an equal volume of acid mercuric nitrate solution, mercury dissolved in twice its weight of nitric acid and this solution diluted to 25 times its volume with water. Shake the mixture, add 20 cc. of water, stir again, allow to stand 5 minutes, and filter. If much gelatin is present, the filtrate will be opalescent and cannot be obtained clear. To a portion of the filtrate contained in a test tube, add an equal volume of saturated aqueous picric acid solution. A yellow precipitate will be produced in the presence of any considerable amount of gelatin, whereas smaller quantities will be indicated by a cloudiness. In the absence of gelatin, the filtrate will remain perfectly clear.

Sour cream, cultured milks, and sour dairy products and those made with rennet invariably give precipitates with picric acid when the above detailed test is performed. The A. O. A. C. recommends that the characteristics of the gelatin-picrate precipitate be taken into consideration in deciding whether or not gelatin is present. The gelatin-picrate precipitate is a fine granular one which does not settle rapidly, whereas that due to rennet and proteins is a flocculent, rapidly precipitating type.

Jacobs and Jaffe Method.[33] In view of the limitations of the Stokes method, these investigators devised a different method for the detection of gelatin. Basic lead nitrate is used as a protein precipitant and calcined charcoal is used to adsorb the pseudo-gelatins formed in the souring process. The basic lead nitrate reagent consists of two solutions added separately to the solution or mixture to be clarified.

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32 Stokes, *Analyst* 22, 220 (1897).

33 Jacobs and Jaffe, *Ind. Eng. Chem., Anal. Ed.* 4, 418 (1932).

To 10 cc. of milk or milk product, add 3 cc. of lead nitrate solution, 250 g. lead nitrate dissolved in water and made up to 500 cc., and stir. Add 3 cc. of sodium hydroxide solution, 25 g. sodium hydroxide dissolved in water and made up to 500 cc., and stir. Add 5 cc. of water and stir, add 0.1 g. of calcined charcoal and stir thoroughly, allow to stand for 5 minutes and filter. To 3 cc. of the filtrate add 2 drops of nitric acid and then a few drops of freshly or recently prepared 5 per cent tannic acid solution. In the presence of gelatin there is a white or brownish voluminous precipitate. In the absence of gelatin the solution remains perfectly clear. As a confirmatory test, add to a portion of the filtrate (no addition of nitric acid is now necessary) an equal volume of freshly filtered saturated aqueous picric acid solution. In the case of considerable quantities of gelatin, there is a heavy precipitate of gelatin-picrate. In the case of smaller quantities, there is a turbidity which develops within 2 minutes. In the absence of gelatin, the filtrate will remain perfectly clear even on standing. For the tannic acid test the addition of nitric acid is essential, for otherwise tannic acid will always give a precipitate. For the picric acid test there is no need to use acid. The addition of acid in this case reduces the sensitivity of the test, because gelatin-picrate is somewhat soluble in nitric acid.

The basic lead nitrate test will give a good test for gelatin for one part of gelatin in 2,000 of the milk product. The test is thus less sensitive but more definite than the official or Stokes method. The Jacobs and Jaffe method gives a precipitate with milk products made with rennet as does the Stokes procedure. Richardson and Tarassuk [34] have modified both methods so that this difficulty is overcome by the use of trichloroacetic acid as a secondary protein precipitant.

Richardson and Tarassuk Method. To 10 cc. of the filtrate obtained from the Stokes or the Jacobs and Jaffe procedures or to an aliquot of these filtrates add 1/2 volume of saturated picric acid solution. Observe the mixture for clearness and type of precipitate. Chill the remainder of the filtrates and add 1/2 volume of 20 per cent trichloroacetic acid solution, shake well and allow to stand at 8–10° C. for about 16 hours, with occasional shaking particularly during the early part of the period. Observe the filtrate-trichloroacetic acid mixture for clearness and type of precipitate. Filter cold, using a medium fast filter paper. Add 1/2 volume of saturated picric acid to this last filtrate. Observe. The purpose of observing carefully lies in the explanation given by Rich-

[34] Richardson and Tarassuk, *J. Assoc. Official Agr. Chem.* 17, 317 (1934).

ardson and Tarassuk that the gelatin-picrate precipitate is a fine granular one, which does not settle rapidly. That due to rennet and proteins is a flocculent, rapidly settling type. These tests may be used and should be used to supplement each other and thus definitely prove the presence or absence of gelatin.

Starch—This substance is probably seldom used as a thickening agent and may be easily detected by the starch-iodine reaction. If the amount of starch used is small, the blue color may be masked and the test should be performed on an acetic acid or preferably a trichloroacetic acid serum of the milk product made by adding 1 cc. of 40 per cent trichloroacetic acid to 10 cc. of milk product or 1 cc. of glacial acetic acid to 100 cc. of diluted sample, allowing to stand and filtering after the curd has settled. The iodine solution is added to a portion of the filtrate and the color produced is observed.

Sucrate of Lime or Sucrose—*Lythgoe*[35] *Method.* In this method any sucrose present is inverted by acid to form dextrose and levulose whereas the lactose present is hydrolyzed to dextrose and galactose. Levulose being a ketose will preferentially reduce the molybdate reagent. It is interesting to note that the ketose reagent of Fischl, which is detailed in Chapter IX, will also be reduced if levulose is present.

To 25 cc. of cream add 10 cc. of 5 per cent uranium acetate solution, shake well, allow to stand for 5 minutes and filter. To 10 cc. of the clear filtrate or to as much of the filtrate as is obtained, add a mixture of 2 cc. of saturated ammonium molybdate solution and 8 cc. of dilute hydrochloric acid, 1 volume of acid of 1.12 sp. gr. to 7 volumes of water, and place in a water bath at 80° C. for 5 minutes. If sucrose is present the solution will be of a prussian blue color, which should be compared with the standard prussian blue color solution. This is prepared by adding a few drops of potassium ferrocyanide and 5 drops of 10 per cent hydrochloric acid to 20 cc. of water containing 1 cc. of a 0.1 per cent ferric chloride solution. Occasionally a sample of pure milk will be found which will give an apparent test but to a much less degree than the standard. Moreover, the color in this case can be removed by filtration, leaving a green filtrate, while the color due to sugar or sucrate of lime is not thus removed.

Seliwanoff Resorcinol Reaction. To 5 cc. of Seliwanoff reagent which

[35] Lythgoe, U. S. Dept. Agr., Bur. Chem., Bull. No. 132 (1910).

is prepared by dissolving 0.05 g. of resorcinol in 100 cc. of dilute hydrochloric acid (1:1) in a test tube, add 1 cc. of a hydrochloric acid serum of the cream or milk product, made by adding hydrochloric acid to milk, allowing to stand and filtering. Heat to boiling preferably in a boiling water bath. A positive reaction is indicated by the production of a red color and the separation of a brown-red precipitate which is soluble in alcohol with the formation of a striking red color.

Gums. The use of the common gums such as tragacanth, karaya, etc., as thickening agents and binders for dairy products has increased a great deal especially in ice cream and cheese products. In cream such substances would obviously be adulterants. A complete schematic method for their identification is detailed in the chapter on gums, Chapter X. They may be detected by one of the following methods. These methods depend on the precipitation of proteins by some protein precipitant which does not affect gums, filtering the gum solution and then precipitating the gum with alcohol. These methods should be used to supplement one another.

Patrick[36] *Method.* To the sample containing the gum, add half its volume of water and boil for a few minutes. Then add 2 cc. of 10 per cent acetic acid for every 50 cc. of sample, heat to boiling and add 3 teaspoonfuls of kieselguhr for every 50 cc. of the mixture. Filter on a plaited filter and discard the precipitate. Precipitate the gum from the filtrate by the addition of 12 cc. of 95 per cent alcohol for every 3 cc. of filtrate. Add 3 cc. of a mixture of 95 cc. of 95 per cent alcohol and 5 cc. of concentrated hydrochloric acid for every 3 cc. of filtrate. The acidified alcohol dissolves the milk proteins that have not been previously precipitated. A flocculent or stringy precipitate insoluble in the acid-alcohol mixture and remaining insoluble upon the further addition of 3 cc. of water for every 3 cc. of filtrate shows the presence of gums.

Jacobs Method. To 10 cc. of the sample or to 10 cc. of a paste of the sample and water add 1 to 2 cc. of 40 per cent trichloroacetic acid solution. Allow to stand for 5 minutes, centrifuge and filter or filter directly as desired. If the filtrate does not come through clear, pass through the filter again. To 2 cc. of the filtrate add 10 cc. of 95 per cent alcohol, stopper and shake. Add a drop of methyl red indicator solution and then 2 to 3 drops of strong ammonium hydroxide solution or sufficient to make the mixture alkaline. Stopper and shake, wait for the protein precipitate

[36] Patrick, U. S. Dept. Agr., Bur. Chem., Bull. 116 (1908).

to develop. Add 2 to 3 drops of hydrochloric acid and mix. A flocculent or stringy precipitate persisting at this point indicates the presence of gums in the original sample.

All of the gums are soluble in the trichloroacetic acid solution. Some of the split proteins are also soluble. Some of the gums are precipitated on the addition of the alcohol. After neutralization with ammonium hydroxide, all of the gums and the split proteins are precipitated and on the addition of the hydrochloric acid, all of the proteins are dissolved leaving only the gums as the precipitant. Gelatin, some starches and dextrins are also soluble in the trichloroacetic acid mixture. They are also insoluble upon the addition of ammonium hydroxide and the further addition of hydrochloric acid does not dissolve them. The separation and distinction of gums from these substances is discussed in the chapter on gums, Chapter X.

Reconstituted Cream

An extended discussion of the reconstitution and homogenization of milk was given in this chapter. Most of the discussion applies equally well to cream. That is, the taste, odor, appearance, sediment, cream line after dilution with water and centrifuging and the so-called added color may all be altered by reconstituting. Hence, the alteration of these properties serves as means for the detection of reconstructed cream.

Evenson [37] Method—The details of this method for re-made cream is somewhat different than in milk because the fat of the cream must be removed. To 15 cc. of cream add 15 cc. of water and warm to 35° C. Precipitate the curd with 2 cc. of 10 per cent acetic acid. Filter and wash. Remove the fat by washing first with 25 to 40 cc. of 95 per cent alcohol and then with 100 to 150 cc. of ethyl ether. Wash thoroughly with water. Test for complete removal of lactose by applying the Molisch test. Add a few drops of the Molisch reagent, a 15 per cent alcoholic solution of α naphthol to a small portion of the wash water and underlay with sulfuric acid. A pink to violet coloration shows the presence of carbohydrate. Continue washing with water until the carbohydrate test is negative. Place the washed curd in test tubes, vials or small beakers and add 10 cc. of 5 per cent sodium hydroxide solution. A yellow coloration of the curd indicates a re-made cream. It is best to run a control on known samples of cream.

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37 Evenson, *Intern. Assoc. Dairy Milk Inspectors, Ann. Rep.* 12, 354 (1923).

Harral [38] uses the formaldehyde titration of milk proteins to detect reconstituted cream prepared from dried milk. The nitrogen content and the formol titration of a sample are estimated. The ratio of nitrogen to formol titration is calculated. The ratio is highly increased by reconstitution due to the destruction of available amino acids.

Letzig [39] detects thickening agents and possible reconstruction by use of the viscosimeter. The addition of pectin and other thickening agents produces an increase in the relative viscosity of the serum over the normal upper limits of these products.

If 5 cc. or 5 g. of cream are shaken with 5 cc. of a mixture of equal parts of benzene and alcohol and the mixture is centrifuged for a short period, reconstituted cream will throw up its butter fat as an amber upper layer, whereas fresh cream will remain as an emulsion with no appreciable separation of the fat layer. After strong centrifuging, a reconstituted mixture shows three distinct layers, while on the other hand, a fresh cream mixture shows only two layers, namely, the aqueous lower layer and an upper opaque stratum.[40]

If, instead of the aforementioned solvents, acetone is used, the following results are obtained: [41]

1) Natural cream gives no definite separation.
2) Artificial cream made from
   a) new milk yields an extensive clear lower layer.
   b) skimmed dried milk yields four layers consisting of a small amber upper layer, a lower opaque stratum, a clear liquid with some coagula, and a little sediment as the bottom or fourth layer.

It is best to run controls with fresh cream side by side with the sample being tested.

Foreign Fat

An imitation cream may sometimes be made from foreign fat, such as coconut oil, hydrogenated fats, etc., and sometimes the fat content of ordinary cream may be raised by the addition of such materials. These

[38] Harral, *Analyst* 58, 604 (1933).
[39] Letzig, *Z. Untersuch. Lebensm.* 72, 312 (1936).
[40] Richardson, *Analyst* 53, 335 (1928).
[41] Richardson, *Analyst* 58, 686 (1933).

practices are considered adulterations and they may easily be detected by an examination of the fat of the sample.

If the cream is sweet, allow it to sour and filter through a large coarse filter with the addition of an equal volume of water. When drained, scrape off the curd and fat from the filter paper and transfer to a beaker. Place the beaker in an oven thermostatically controlled at 100 to 105° C. until the curd chars and the fat is dry. Filter the fat through cotton in a small funnel into a suitable container and then proceed as directed in the chapter on fats and oils, Chapter VIII. Sour cream may be filtered directly with the aid of additional water.

Preferentially dry the curd and fat in a vacuum oven, transfer to a continuous extractor, extract the fat with ether, evaporate off the ether and dry the fat at 70 to 75° C. in an oven thermostatically controlled. Then proceed with the examination of the fat.

REFERENCES

Davies, "Chemistry of Milk," Van Nostrand (1936).
Mojonnier and Troy, "Technical Control of Dairy Products," Mojonnier Bros. (1925).
Woodman, "Food Analysis," McGraw-Hill (1931).
Leach-Winton, "Food Inspection and Analysis," Wiley (1920).
Allen's Commercial Organic Analysis, 5th ed., Vol. IX, Blakiston (1933).
Rogers, "Fundamentals of Dairy Science," Reinhold, 2nd ed. (1935).
Eckles, "Daily Cattle and Milk Production," Macmillan (1918).
Ling, "Textbook of Dairy Chemistry," Wiley (1930).
Burke, "Practical Dairy Tests," Olsen (1935).
Eckles, Combs, and Macy, "Milk and Milk Products," McGraw-Hill (1936).
Methods of A. O. A. C. (1935).
Standard Methods of Milk Analysis, Am. Pub. Health Assoc., 6th ed. (1934).
Standard Methods of Water Analysis, Am. Pub. Health Assoc., 8th ed. (1936).
Wolf, "The Human Fuel," Chapman and Grimes (1936).

CHAPTER VII

MILK PRODUCTS

BUTTER

When cream is churned the fat droplets coalesce and form progressively larger clusters of fat globules. These grains finally break away from the surrounding liquid and form a semi-solid substance called butter. The definition of the Food and Drug Administration, United States Department of Agriculture is, butter is the food product usually known under that name. It is made exclusively from milk or cream, or both, with or without the addition of common salt and with or without the addition of coloring matter.

It is generally sold as salt or sweet butter and in the form of tub, cube, print or whipped butter. Whipped butter is a more recent departure in butter making and is the product of the process of incorporating air into the butter by means of mechanical agitation. This process makes the butter appear fluffy and creamy. Since butter is sold by weight, the incorporation of air makes little difference in contradistinction to ice cream which is sold by volume and in which product the incorporation of air makes a large difference.

The chief component of butter is, of course, butter-fat or milk-fat and comprises at least 80 per cent of the food material. It is one of the chief articles of our diet and is, hence, an important one. Adulteration of butter consists in the main of adding water and thus lowering the butter-fat content, adding a foreign fat, renovating old and rancid butter and then selling this product as fresh butter, adding flavor in order to make the butter appear better than it really is. All of these forms of adulteration and sophistication are susceptible to chemical detection.

Moisture

Weigh 3 g. of the properly prepared sample into a tared, low, flat-bottomed dish and place in a thermostatically controlled oven at 100 to 105° C. for 4 hours. At the end of this period place the dish in a desiccator and when cool, weigh. The loss in weight divided by the weight of the original sample taken for analysis is deemed to be moisture.

Fat by Difference

Prepare a Gooch crucible with a thin pad of asbestos. Wash, dry, ignite, cool and weigh. Transfer the contents of the dish from the moisture determination to the Gooch with the aid of a wash bottle containing petroleum ether, maintaining suction on the crucible during the washing. Carefully transfer all of the curd and salt to the crucible with the aid of the ether. Continue washing with the ether until all of the fat has been washed out. Suck dry, place in the oven for one hour. Remove the crucible, place in a desiccator and weigh when cool. The increase in weight is the curd plus salt. The percentage of moisture plus the percentage of curd and salt subtracted from 100 gives the percentage fat.

Fat

The percentage fat may be determined directly by placing the dish and contents from the moisture determination in a Soxhlet continuous extractor or some similar apparatus, extracting the fat with petroleum or ethyl ether, receiving the ether in a tared flask and then evaporating off the petroleum or ethyl ether extract. The flask is dried, cooled and weighed. The increase in weight is calculated as the percentage fat.

Salt

Weigh 10 g. of butter into a beaker. Transfer with the aid of hot water to a separatory funnel. Allow the layers to separate and draw off the water layer into a flask. Do not allow any of the fat to pass through the funnel. Repeat the extraction 10 to 15 times with 20 cc. of hot water and collect the extractions in the same flask. Rinse the original beaker with each portion of wash water before adding to the separatory funnel. Titrate the washings, which will contain practically all of the salt, with standard silver nitrate solution, using potassium chromate as the indicator.

Jacobs Rapid Crucible Method

The moisture, fat, curd plus salt, and salt are all determined on one weighed portion of butter by the use of a specially prepared crucible and technical carbon tetrachloride as a solvent. The method uses a Gooch crucible containing a thin pad of asbestos over which a layer three-

eighths of an inch thick of finely ground alundum is placed. The asbestos pad must be thick enough to prevent any of the alundum from passing through, yet thin enough to allow a free flow of the solvent. The crucible is ignited in a muffle oven and then cooled. It is washed well with water, alcohol, and ether and dried in a constant temperature oven. By using technical carbon tetrachloride as the solvent, the collected washings from the fat-by-difference determinations may be saved. The carbon tetrachloride washings may then be purified by distillation and re-used.

The sample of butter, contained in a mason jar, is softened, but not melted, in a steam oven or by some other suitable means and thoroughly mixed with a long and broad spatula so that a homogeneous mass is obtained. One to 1.5 g. of the butter is accurately weighed into a tared crucible prepared as directed above. The crucible is heated for an hour and a half at 100 to 105° C., placed in a desiccator to cool and weighed at the end of twenty minutes. The loss in weight is calculated as per cent moisture.

The crucible is placed in an eight ounce bottle having a mouth wide enough to hold the crucible and is washed with 160 cc. of carbon tetrachloride. The crucible is filled very carefully almost to the top with the solvent and allowed to drain completely before any more of the solvent is added. Splashing must be avoided, otherwise the results will be vitiated. At the completion of the washing, slight suction is applied to the crucible to drain it as completely as possible. It is then placed in a constant temperature oven and dried at 100 to 105° C. for one hour, placed in a desiccator to cool, and weighed. The gain in weight over that of the crucible, itself, is calculated as per cent curd plus salt. The sum of the per cent moisture and the curd plus salt subtracted from 100 gives the per cent butter fat.

The crucible may now be ignited at just below redness, placed in a desiccator and weighed when cool. The loss in weight may be calculated as casein.

Wash the crucible well with water and catch the washings in a flask. Titrate the salt solution with standard silver nitrate solution using potassium chromate solution as indicator and obtain the percentage of salt.

Foreign Fat

The butter fat may be prepared for examination for adulteration with fats foreign to milk by placing the butter in a beaker and heating the

beaker and its contents in an oven at 100 to 105° C. until it is dry and the curd is charred. Or the butter may be melted in a tall cylinder at about 60° C. for a few hours until the fat and water layers are completely separated and then the supernatant fat is filtered through cotton in a small funnel into a suitable container. The physical and chemical constants of the fat may then be determined as directed in the chapter on oils and fats, Chapter VIII.

Renovated Butter and Oleomargarine

The foam test, also known as the spoon test for distinguishing between butter on the one hand, and renovated butter and oleomargarine on the other, was originally intended as a household test. It is just as indicative in the laboratory. Heat 2 to 3 g. of the sample in either a spoon or a dish over a small flame. True butter will foam copiously, whereas process butter will bump and sputter like hot grease, with little or no foaming. Oleomargarine behaves like process butter, but chemical tests will determine whether the sample is oleomargarine or butter.

Another simple test used to distinguish between true butter and reworked butter and margarine is the following. Melt 50 to 100 g. of the sample at 50° C. The curd from butter will settle, leaving a clear supernatant fat, in the case of renovated butter, the supernatant fat remains more or less turbid.

Biacetyl and Acetylmethylcarbinol

The compound responsible for most of the flavor in butter is biacetyl. Entering into the butter during manufacture are both biacetyl (diacetyl, dimethylketone, 2–3 butandione, $CH_3COCOCH_3$), and acetylmethylcarbinol (acetoin, 3-hydroxy 2 butanone, $CH_3CHOHCOCH_3$), which are natural flavoring components of butter. The biacetyl arises from 2 sources,

1) bacterial action on the acetylmethylcarbinol
2) auto-oxidation of the same compound.

Well ripened cream, that is of 0.6 per cent lactic acidity from a good starter, contains 5 to 10 ppm. of biacetyl and 100 to 200 ppm. of carbinol. A starter is a pure bacterial culture used to initiate an industrial fermentation. However, the resulting butter will contain of these substances, calculated as biacetyl, less than 1 to 2 ppm. in slightly flavored butter [1]

[1] Davies, *Dairy Ind.* 1, 165 (1936).

and as much as 4 ppm. in very highly flavored butter. A greater content of total biacetyl may be viewed with suspicion.

Butter that has an off taste and odor is sometimes reflavored by the addition of biacetyl. This is an adulteration, according to the definition of butter, because nothing except salt or color may be added. This addition may be done during the process of whipping, that is, during the process of incorporating air into the butter.

Vizern and Guillot[2] Method—Vizern and Guillot give the following method for the detection of biacetyl. To 50 g. of sample in a round-bottom, 250 cc. distillation flask, add 20 cc. 95 per cent alcohol, insulate the neck of the flask to avoid excessive fractionation. Distill by immersing the flask in a calcium chloride bath at 115 to 120° C. and using a vertical condenser, collect 20 cc. of the distillate. Transfer the distillate to a porcelain dish and rinse the receiver with 5 cc. of water. Add successively 1 cc. of 10 per cent hydroxylamine hydrochloride and 1.7 cc. of *N* aqueous sodium hydroxide solution. Stir for 1 minute. Add 1 cc. of 10 per cent nickel sulfate solution and then 0.6 cc. of *N* acetic acid, drop by drop with constant stirring. Evaporate the alcohol on a water bath. In the presence of biacetyl, a characteristic red zone of nickel dimethylglyoxime adheres to the dish when the contents of the dish have been reduced to about 2 to 3 cc. According to Vizern and Guillot, pure butter, free from improver gives a negative test but according to more recent investigations pure butters do have a slight amount of biacetyl as noted above.

This test may be made more sensitive and far more characteristic by continuing the evaporation to dryness. Then extract the residue with 3 successive portions of chloroform and filter each extraction through a small filter, catching the filtrate in another porcelain dish. Evaporate to dryness either spontaneously or on a water bath at low temperature. If biacetyl was present in the original sample, a series of red rings due to nickel dimethylglyoxime will be found in the porcelain dish. With continued experience the approximate amount of biacetyl may be estimated from the depth of color in the porcelain dish due to the rings.

Modified Barnicoat Method—The following Barnicoat[3] method, modified by Jacobs, may be used to determine the amount of biacetyl

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[2] Vizern and Guillot, *Ann. fals.* **25**, 45 (1932).

[3] Barnicoat, *Analyst* **60**, 653 (1935).

quantitatively in butter and like products. Weigh 400 g. of butter into a liter florence flask or better into a liter flask equipped with a ground glass stopper. The stopper has two tubes arranged for steam distillation one of which tubes has a Polenske or other trap attachment. Place the flask into an oil bath and add 75 cc. of 0.1 *N* sulfuric acid and enough salt to saturate the solution. It is best to have the flask almost completely immersed in the oil at a temperature of 115° to 120° C. Connect the flask with a source of steam and to an upright condenser by means of a Polenske trap or with the above mentioned ground glass connections. Have the tip of the condenser dip into or have an adapter connected to the condenser dip into a mixture of 4 cc. of 20 per cent hydroxylamine hydrochloride, 4 cc. of 20 per cent sodium acetate and 2 cc. of 5 per cent nickel sulfate. Steam distill until 150 to 200 cc. has been carried over, stirring the distillate and the fixing solution occasionally. Evaporate the distillate on a hot plate or steam bath at 80° C. until a volume of 10 cc. is obtained. Allow to stand and cool. Transfer the remaining solution and precipitate to a small separatory funnel, washing the vessel in which the evaporation was performed with two 5 cc. portions of water. Extract the nickel dimethylglyoxime with 4 successive portions of chloroform, 15 cc. for the first portion and then with 10 cc. portions. Wash the vessel in which the evaporation was carried out with each successive portion of chloroform before transferring to the separatory funnel. Allow each chloroform extract to separate completely and then draw off the lower layer passing it through a very small filter, catching the filtrate in a flat-bottomed crystallization dish or small evaporating dish that has been previously dried and weighed. After the four portions of chloroform have passed through the filter paper wash the filter with one more 5 cc. portion of the solvent. Evaporate the chloroform spontaneously or on a water bath at low heat. Dry in a thermostatically controlled oven at 100 to 105° C. for an hour, place in a desiccator to cool and weigh. The weight is due to nickel dimethylglyoxime and the various relationships may be computed as follows:

1) Nickel dimethylglyoxime × 0.596 equals biacetyl

2) Nickel dimethylglyoxime × 0.610 equals acetylmethylcarbinol

3) Distillation with ferric chloride yields both biacetyl and acetylmethylcarbinol and is computed as total biacetyl

4) Distillation in carbon dioxide atmosphere yields only biacetyl.

5) The results (3) minus (4) gives acetylmethylcarbinol

The reactions taking place are the following:

$$CH_3COCOCH_3 + H_2NOH \rightarrow CH_3\overset{\overset{NOH}{\|}}{C}-COCH_3 + H_2O$$

$$CH_3\overset{\overset{NOH}{\|}}{C}-\!\!-COCH_3 + H_2NOH \rightarrow CH_3\overset{\overset{NOH}{\|}}{C}-\!\!-\!\!-\overset{\overset{NOH}{\|}}{C}CH_3 + H_2O$$

$$2CH_3-\overset{\overset{NOH}{\|}}{C}-\!\!-\overset{\overset{NOH}{\|}}{C}-CH_3 + NiCl_2 \rightarrow [\ CH_3\overset{\overset{NOH}{\|}}{C}-\!\!-\overset{\overset{NO}{\|}}{C}-CH_3\ ]_2\ Ni + 2HCl$$

The nickel dimethylglyoxime forms a yellow solution in chloroform and may be estimated colorimetrically by comparing with known amounts of the nickel organic compound dissolved in the same solvent. Nickel dimethylglyoxime is also soluble in tetrachloroethane which may be used as the solvent in colorimetric determinations.

Microscopic Examination and Extraneous Matter

Microscopic examination of butter will show the presence of crystals of other fats if these have been added to the butter. Renovated butter will also show crystals and variegated colors with a selenite plate. If the butter has been made from milk or cream taken from unclean farms or dairies, a microscopic examination will show the presence of dirt, rat hairs and other contaminants.

Weigh into a beaker 100 g. of butter and add 150 to 200 cc. of borax solution, 40 g. of borax in 1 liter of water. Heat to boiling and filter through a Büchner funnel containing a 7 cm. rapid filter paper [4] supported by a 50 mesh copper screen 6 cm. in diameter. Wash well with gasoline or petroleum ether to remove fat. Wash with hot water. Examine the filter paper under a wide field microscope, Fig. 45, for foreign material. The debris may be transferred to a microscope slide having a drop of glycerol and examined under a compound microscope for identification. If the butter is contaminated with much mold, the filter paper will clog very rapidly. In this case, stop the filtration, wash the paper with the aforementioned solvents. Remove the paper, replace with another sheet and continue the filtration. Examine each sheet as detailed.

Butter churned from decomposed cream generally retains some of the

[4] Greene, *Food Industries* 7, 442 (1935).

decomposition products. Among these are increased acidity, higher aldehydes that respond to the Kreis test, indole, and mold. Clarke[5] and

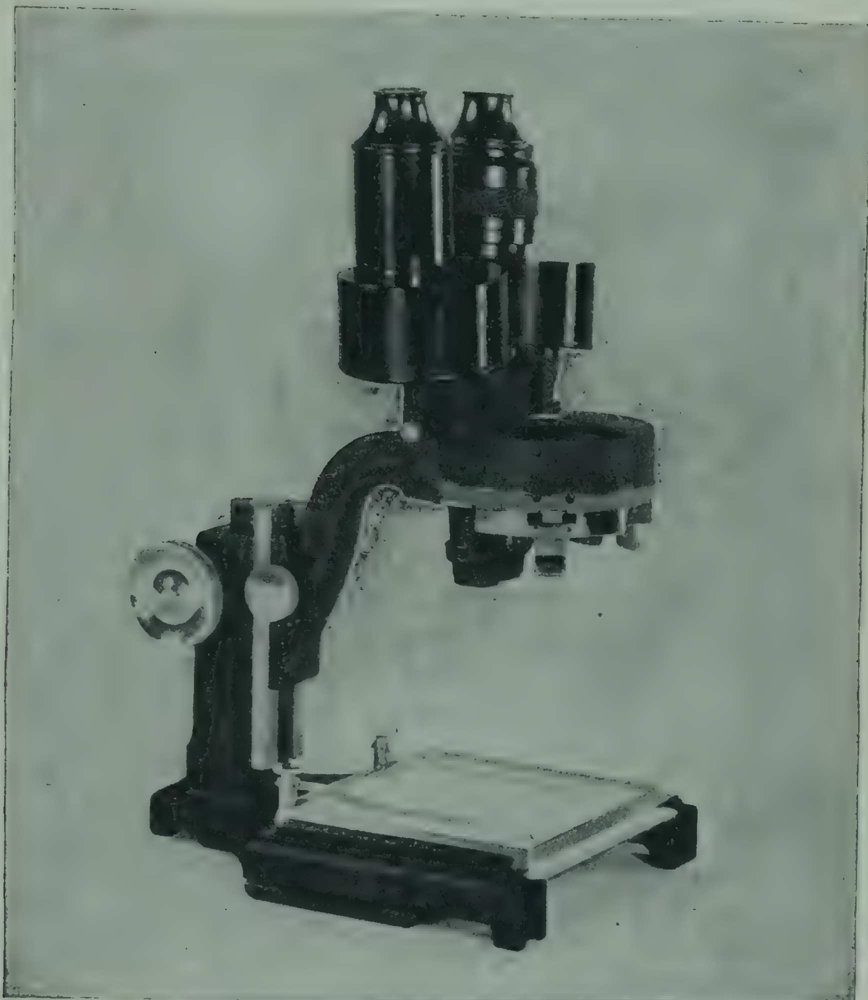

Fig. 45. Wide Field Microscope.. (Courtesy of Spencer Lens)

coworkers discuss this subject fully and give procedures for the detection of these decomposition products.

CHEESE

Cheese is the food product made from the separated curd obtained by coagulating the casein of milk, skimmed milk, or milk enriched with

[5] Clarke, *J. Assoc. Official Agr. Chem.* 20, 475 (1937).

cream. The coagulation is accomplished by means of rennet or other suitable enzyme, lactic fermentation, or by a combination of the two. The curd may be modified by heat, pressure, ripening ferments, special molds, or suitable seasoning. Certain varieties, such as for example Roquefort cheese, which is made from the milk of sheep, are made from the milk of animals other than the cow. Cheese unqualified generally means American Cheddar cheese. There are hundreds of names for cheese, native and foreign, although there are only about 18 varieties. These may all be classified under the headings, whole milk cheese, part skim milk cheese, skim milk cheese, pasteurized cheese and process cheese, or as hard or soft curd cheese.

The classifications other than process cheese are self explanatory. In this generation, the processing of cheese has become a large industry. Process cheese is the modified cheese made by comminuting and mixing one or more lots of cheese into a homogeneous, plastic mass, with the aid of heat, with or without the addition of water and with the incorporation of not more than 3 per cent of a suitable emulsifying agent. The name process cheese unqualified applies to process Cheddar cheese and those with a varietal name correspond to the process variety indicated by that name. Of course, these types of cheese must conform to the limits set for the variety of cheese.

The following table 7 gives the percentage composition of some of the more well known varieties of cheese.

Cheese may be adulterated in many ways. It may be substandard in the limits set for fat and protein. It may contain excessive amounts of water and foreign fat. It may, if it be a process cheese, contain excessive amounts of water and emulsifying agent or binder. Many times cheese is misbranded not only as to brand name but also as to method of processing. These adulterations and misbranded statements are generally capable of detection by chemical methods. Some of these will be detailed.

Moisture

Weigh 4 to 5 g. of the properly prepared sample into a tared flat-bottomed dish containing a small stirring rod and sand. Rub the cheese and sand carefully together and place the dish in a constant temperature oven overnight at 100 to 105° C. Remove the dish from the oven, place in a desiccator to cool and weigh. The loss in weight is calculated as per cent moisture.

TABLE 7. COMPOSITION OF VARIETIES OF CHEESE

Variety	water	fat	protein	ash
Brick	42.5	30.7	21.1	3.0
Caciocavallo	35.0	22.0	34.3	7.0
Camembert	47.9	26.3	22.2	4.1
Cheddar	36.8	33.8	23.7	5.6
Cottage	69.8	1.0	23.3	1.9
Cream	42.7	39.9	14.5	1.9
Edam	38.1	22.7	30.9	6.2
Emmenthaler	33.0	30.5	30.4	4.2
Gorgonzola	37.3	34.7	25.2	3.8
Gouda	38.1	24.5	29.6	6.1
Gruyere	30.0	28.2	33.0	4.0
Limberg	54.8	19.6	21.3	5.2
Munster	40.6	31.0	22.2	4.6
Neufchatel	52.1	23.5	19.3	5.0
Parmesan	17.0	22.7	49.4	7.6
Pecorino	29.8	30.5	33.5	6.2
Romano	29.6	27.7	31.2	8.7
Roquefort	38.7	32.2	21.4	6.1
Sapsago	47.8	2.0	41.6	11.9
Stilton	33.6	31.2	29.0	3.0
Swiss	33.9	30.6	29.2	4.2

The analyses given in the table have been abstracted from U. S. Dept. Agr. Bull. No. 608, "Varieties of Cheese" (1932). No typical analyses are given for process cheese and for filled cheese. These should follow closely the varietal name.

FAT

Weigh accurately into the lower section of a Jacobs-Singer separatory flask about 1 g. of the sample and add 9 cc. of water and 1 cc. of ammonium hydroxide. Warm on a steam bath or hot plate, regulated at a low temperature. Stir by shaking until the curd is completely softened. Add ½ cc. of hydrochloric acid and stir. Add 10 cc. more of hydrochloric acid and a pinch of sand. Boil the mixture gently for 5 minutes. Cool the mixture and stopper with the upper section of the separatory flask. Add water to the middle of the connecting joint, and shake. Add 25 cc. of ethyl ether and after stoppering the flask, shake thoroughly. Add 25 cc. of petroleum ether, again shaking vigorously. Allow the layers to separate and draw off the ether layer into a tared fat flask. From this point proceed as directed in the Roese-Gottlieb method for the determination of fat from the point of drawing off the ether layer into a tared fat flask.

As an alternative procedure, weigh into a tall form 100 cc. beaker, 1 g. of the sample and add 9 cc. of water and 1 cc. of ammonium hydroxide. Warm on a hot plate, stirring with a glass rod, until the curd is completely softened. Add ½ cc. of hydrochloric acid and stir. Add 10 cc. more of hydrochloric acid and a pinch of sand. Cover the beaker with a watch-glass on glass hooks and boil the mixture gently for 5 minutes. Cool the mixture and transfer to a Mojonnier tube or to a Jacobs-Singer separatory flask. Rinse the tall form beaker with sufficient water to bring the level of the water up to the middle of the constriction in the Mojonnier extraction tube or the middle of the connecting joint of the Jacobs-Singer separatory flask. Then rinse the beaker and watch glass and glass hooks with 25 cc. of ethyl ether and add the washings to the extraction tube or flask. Stopper and shake thoroughly. Repeat with 25 cc. of petroleum ether. From this point proceed as directed above.

FIG. 46. Gerber Cheese Butyrometer and Scoop

Gerber Method—The fat of a soft curd cheese may easily be determined by the following modification of the Gerber method. Weigh or counterpoise a scoop, Fig. 46, held in a one hole rubber stopper and weigh into the scoop exactly 2.5 g. of the cheese. Place the scoop into a cheese butyrometer, Fig. 46, and add 9 cc. of 4 per cent borax solution and 1 cc. of amyl alcohol. Heat to 70° C. until the curd is completely disintegrated. Cool slightly and add carefully 10 cc. or less if necessary of sulfuric acid, sp. gr. 1.82. Insert the small stopper and then mix thoroughly by inverting the butyrometer several times. Whirl for 10 minutes at the proper speed and then remove and read the fat percentage directly as directed under milk and cream. The specifications for the cheese butyrometer are also rigidly drawn.[6]

[6] Dept. Agr. Markets, State of New York, Circ. No. 515 (1936).

Protein

The protein content may be estimated by determining the nitrogen content in an accurately weighed portion of the cheese, as directed in the Kjeldahl-Gunning-Arnold method given in Chapter I. Weigh 2 to 3 g. of the cheese on ashless filter paper as described in the above mentioned method and then the contents and filter paper are transferred to the Kjeldahl flask or weigh the cheese by difference into the flask from a weighing bottle. The percentage of nitrogen found multiplied by the factor, 6.38, equals the percentage of protein.

Other Constituents

Ash, preservatives, coloring matters, acidity and chlorides may be estimated in the usual manner. For the determination of tartaric acid, citric acid, and phosphates that are used as emulsifying agents in the manufacture of process cheese, refer to the appropriate chapters. For the estimation of lactose, refer to Chapters VI and IX. For the detection of gums in cheese, the reader may use one of the methods outlined under cream or in the chapter on gums, Chapter X. The fat may be extracted for examination in a manner entirely analogous to the one detailed in the following section concerning ice cream.

Extraneous Matter

Cheese that is made from dirty milk or cream or that is made under unsanitary conditions is likely to contain extraneous matter. This foreign matter is usually present in very small particles. In process cheese where a grinding process is part of the method of manufacture, particles of foreign matter are especially likely to be small. The examination of cheese is more difficult than that of butter because of

1) the use of rennet for precipitating the casein,

2) the variable methods used for manufacturing cheese, especially process cheese in which organic substances, inorganic substances, and gums are used as emulsifying agents, and

3) the presence of a large number of bacteria.

Greene [7] devised the following procedure. Weigh 50 g. of cheese, excluding the rind, and place in a mortar. Add 100 cc. of a freshly

[7] Greene, *Food Industries* 7, 442 (1935).

filtered solution of 950 cc. of 95 per cent alcohol plus 50 cc. of hydrochloric acid, sp. gr. 1.18, slowly while stirring the cheese to a smooth paste. Pour the paste into a beaker rinsing the mortar with another 100 cc. portion of the solution. Heat slowly and allow to boil gently for 3 minutes. Filter at once through a 7 cm. rapid filter paper placed in a Büchner funnel. The filter paper should be supported by a piece of 50 mesh copper screen 6 cm. in diameter. Wash the filter immediately with ½ liter of previously filtered boiling water until the paper shows no cheese material left. The filter paper is then examined with a wide field binocular, Fig. 45, to detect the extraneous material. For exact identification, the debris may be examined by transferring it to a drop of glycerol on a microscope slide and looked at with a compound microscope.

Fragments of insects, principally flies or other insects and larvae, rodent and other hairs, paper, wood, and nondescript debris may be found and identified by this method.

VITAMIN D MILK

Vitamin D milk was first introduced in 1932. In 1936, about 400,000,000 quarts were produced which was approximately three per cent of the total volume of household fluid milk. There are a number of different types marketed. One type, namely, irradiated milk, is produced through direct irradiation of milk. Since milk is known to contain cholesterol, irradiated milk has the cholesterol type vitamin D. Another type is metabolized vitamin D milk. It is produced by feeding the cow irradiated yeast which is known to contain ergosterol, hence this milk has the ergosterol type vitamin D. In view of the basic difference in types of vitamin D in the milk one should expect a difference in potency of these milks when tested on rats and on chickens. Various investigators have shown that metabolized milk has approximately one-tenth the antirachitic effect of the irradiated milk when fed to chicks on an equal rat unit basis.

As marketed in 1936 they had 135 U. S. P. units for the irradiated, and 430 U. S. P. units per quart for the metabolized milk. Hence irradiated milk is approximately one-third as effective as a source of vitamin D in the prevention and cure of rickets as metabolized milk. There is no practical difference between the two milks, unit for unit, in respect to their effectiveness for rachitic infants. Hence metabolized milk has 3 times the antirachitic effect as irradiated milk when fed on an equal

volume basis. Refer to Chapter XVI for the value and interpretation of these units.

Another type of vitamin D milk, fortified milk, is made by adding to milk a vitamin D concentrate from cod liver oil. The potency of fortified milk is adjusted by producers at approximately 400 U. S. P. units per quart and it contains the cholesterol type of vitamin D.

In the chapter on vitamins, Chapter XVI, vitamin D and its estimation is more fully discussed.

EVAPORATED MILK

Evaporated milk is the product resulting from the evaporation of a considerable portion of the water from milk, or from milk with the adjustment, if necessary, of the ratio of fat to non-fat solids by the addition or the abstraction of cream. It contains not less than 7.8 per cent of milk fat, nor less than 25.5 per cent of total milk solids; provided, however, that the sum of the percentages of milk fat and total solids be not less than 33.7. After dilution with water the sample may be analyzed in a manner similar to milk with, of course, a correction for the dilution in calculating results.

Scrape, if necessary, the contents of the can into a dish large enough to hold all the sample, and mix sufficiently to make the sample homogeneous. If the material has separated out, the sample may be warmed and passed through a hand pressure colloidal mixer, commonly known as a mechanical cow. Weigh 40 g. into a flask and add 60 g. of water and proceed with the determinations as directed under milk correcting the results obtained for dilution.

Rapid Method for the Analysis of Evaporated Milk

Weigh 40 g. of the properly mixed sample into a beaker and add 80 g. of water. Stir thoroughly until a homogeneous mixture is obtained. Transfer to a cylinder and take the lactometer reading in the usual way at 60° F. Determine the fat in exactly the same way as that detailed in the Babcock method for milk, except that slightly more acid need be added. Calculate the total solids according to one of the formulas given in the section "Rapid Method" under milk. Multiply the fat and calculated total solids results by 3 to obtain the percentage in the original material.

SWEETENED CONDENSED MILK

Sweetened condensed milk is the product resulting from the evaporation of a considerable portion of the water from milk to which sugar or dextrose or both has been added. It contains not less than 28 per cent of total milk solids and not less than 8 per cent of milk fat. The contents of the can is warmed and then scraped into a dish and thoroughly mixed until the mass is homogeneous. Weigh 100 g. of the sample into a 500 cc. volumetric flask, then dilute to the mark with water and mix. The usual determinations are made in a manner similar to that of milk. For the determination of sucrose the reader is referred to the chapter on sugars, Chapter IX. The fat content of evaporated and sweetened condensed milk may be determined by the Gerber method using ice cream butyrometers, Fig. 48. Evaporated milk is weighed directly. Condensed milk is diluted one-half with water before being weighed into the butyrometer.

DRIED MILK

Dried milk is the product resulting from the removal of water from milk. The fat content of this milk product may be estimated by the Gerber method in a manner similar to that of evaporated milk. Weigh accurately the amount of powder taken and the amount of water used to dissolve it and then proceed as directed under evaporated milk, making the proper correction for dilution.

FERMENTED MILK

Milk is fermented in order to preserve it. The fermentation is caused by the lactic acid bacillus, which changes the lactose to lactic acid. Of the many fermented milks prepared and sold, buttermilk is by far the most important. There are two types of buttermilk, artificial and natural. Natural buttermilk represents the liquid residue which remains after the separation of butter. It contains little fat, but on the other hand contains most of the mineral matter and vitamins except vitamin A of milk. Artificial buttermilk is prepared by the addition of lactic bacilli to skimmed milk. Sometimes the lactic acid bacilli culture is added to whole milk or partially skimmed milk. The Food and Drug Administration defintions of these products are:

Buttermilk is the product that remains when fat is removed from milk or cream, sweet or sour, in the process of churning. It contains not less than 8.5 per cent of milk solids not fat.

Cultured buttermilk, is the product obtained by souring pasteurized skimmed or partially skimmed milk by means of a suitable culture of lactic bacteria. It contains not less than 8.5 per cent of milk solids not fat.

Buttermilk may be analyzed in a manner similar to other milk products. Fat can be determined easily by either the Roese-Gottlieb method or the Gerber method. Because of the viscosity only gravimetric determinations of total solids can be made. The other determinations follow the line previously described.

Some of the other types of fermented milk are Kefir, Koumiss, Yogurt, Matzoon and acidophilus milk. The first four contain from 1 to 2 per cent of alcohol. Acidophilus milk results from fermentation with acidophilus bacteria and is very similar to buttermilk in appearance.

MALTED MILK POWDER

Malted milk is the product made by the combination of whole milk with the liquid separated from a mash of ground barley malt and wheat flour, with or without the addition of sodium chloride, sodium bicarbonate, and potassium bicarbonate, in such a manner as to secure the full enzymic action of the malt extract, and by removing water. The manufacture of malted milk powder involves the preparation of a malt-flour infusion by the process of mashing and the evaporation to dryness of the infusion-milk mixture. The ground malt is used to make the malt-flour. Malted milk should contain 7.5 per cent of butter fat as a minimum.

This product may be adulterated by the substitution of skim-milk for whole milk in the aforementioned process, by mixing skim-milk powder with the malted milk powder, by adding starch, sugar and other substances. Many adulterations of malted milk are easily detected by microscopical examination with the aid of comparative photomicrographs identifying malted milk and its allied products. The chemical quality of this product may, of course, be determined by suitable means.

Fat

The fat content of malted milk powder may be estimated by a modified Babcock method. One gram of the powder, accurately weighed is transferred to a tall form 100 cc. beaker containing 5 cc. of water. Stir this mixture thoroughly and then add 5 cc. of 85 per cent lactic acid. Bring the mixture to a boil and continue heating until the powder is

completely dissolved. Transfer to a skim-milk Babcock flask, washing the beaker with a total of 10 cc. of water and adding the washings to the flask, Fig. 47. Add 2 cc. of amyl alcohol or 1 cc. of butyl alcohol and 1 cc. of amyl alcohol. Add 17.6 cc. of a mixture of 200 cc. of commercial sulfuric acid and 30 cc. of water, adding the acid carefully to the water. Stir as in the Babcock milk method and centrifuge for five, two and one minutes, adding hot water at the end of the first and second whirlings as previously described. Read the percentage of fat by assuming full scale reading equals 90 mg., or on basis of 1 g. sample 9 per cent. Therefore each division is equivalent to 0.18 per cent or 1.8 mg. fat. The fat content may also be estimated by the Roese-Gottlieb method.

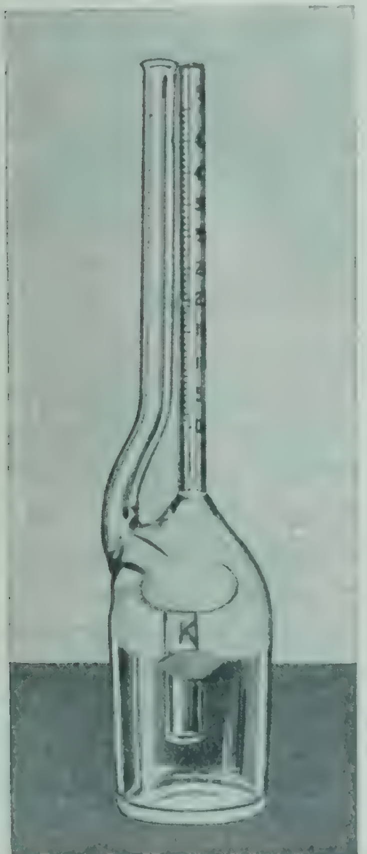

Fig. 47. Babcock Skim Milk Flask

Weigh into the lower section of a Jacobs-Singer separatory flask 1 g. of the powdered sample. Add 11 cc. of water and shake until a thick paste is formed. Add 2 cc. of ammonium hydroxide, boil for 5 minutes and cool. Add 11 cc. of ethyl alcohol and bring the volume of the mixture up to the middle of the connecting joint. Add 25 cc. of ethyl ether and proceed with the method as detailed under milk.

Alternatively weigh 1 g. of the powdered sample into a tall form 100 cc. beaker. Add 1 cc. of water and stir to a thick paste. Add 5 cc. more of water, stir thoroughly, add 2 cc. of ammonium hydroxide, boil for 5 minutes, cool and transfer to a Mojonnier extraction tube or a Jacobs-Singer separatory flask. Wash the beaker with a total of 5 cc. of water and add the washings to the extraction tube or flask. Proceed with the method as detailed immediately above and as under milk using the first portions of the solvent to wash the beaker.

Moisture may be determined by the vacuum oven method in the usual manner, or it may be estimated by drying at a lower temperature for a longer period of time. Protein, ash, carbohydrate, and other constituents may be ascertained by customary means.

Malted Milk Drink

Sometimes it is necessary for the analyst to find out whether or not malted milk powder has been added to a drink sold as a malted milk drink. Malt contains dextrins, for, as the malt is prepared from barley or other grain, higher dextrins, malto-dextrins, etc., must be formed as intermediates in the formation of maltose. Hence maltose and malto-dextrins are characteristic constituents of malted milk powder.

Based on the foregoing characteristic property, the following test for the detection of malted milk powder in malted milk drink was devised by Jacobs. To 10 cc. of the malted milk drink, add 5 cc. of 5 per cent uranium acetate solution, 5 g. $UO_2(OAc)_2.2H_2O$ dissolved in water, made up to 100 cc. and centrifuged. Allow the mixture to stand for 5 minutes after stirring thoroughly. Filter and repass the first 2 cc. through the filter again. To 2 cc. of the filtrate, add 2 drops of hydrochloric acid. Then add 20 cc. of 95 per cent alcohol and mix. The presence of dextrins is indicated by a marked persistent white turbidity. In the case of large amounts, a flocculent precipitate may form. A positive test does not prove the presence of malted milk powder in the drink, for if the drink were made, let us say, with commercial glucose, a positive test for dextrins would also be obtained. A negative test indicates the probable absence of malted milk powder in the drink. Gums and pectins do not interfere because these substances are either precipitable by uranium acetate in the presence of milk or do not yield a white turbidity on the addition of alcohol. Chocolate flavor syrup may interfere because such syrups often contain commercial glucose and dextrins.

ICE CREAM

Ice cream is the frozen product made from a combination of milk products and two or more of the following ingredients: eggs, water, and sugar with harmless flavoring and harmless coloring, with or without stabilizer, and in the manufacture of which freezing is accompanied by agitation of the ingredients. The milk products generally used are one or more of the following: cream, butter, milk, evaporated milk, skimmed milk, condensed milk, sweetened condensed milk, condensed skimmed milk, sweetened condensed skimmed milk, dried milk, dried skimmed milk. Ice cream formerly was considered more nearly a confection than a staple article of our diet but for many people, especially children, it is a regular item of their daily fare. Consequently its manufacture and sale should be as rigidly controlled as is milk.

Ice cream manufacture presents one possible type of adulteration that has not been encountered previously in this text. It is made by agitating while it is being frozen. This process incorporates a great deal of air and increases the volume of the ice cream mix, the term applied to the mixture of ingredients before freezing, usually from 90 per cent to 110 per cent. Since ice cream is sold by volume rather than by weight, it is obvious that it is necessary to limit the incorporation of air. This is generally done by having a minimum value of total food solids per gallon of ice cream. The other types of adulteration are not different from that encountered in the previously described milk products. The addition of yellow coloring matter to ice creams labeled "French ice cream," "Custard," or "Frozen Custard" which are required to contain eggs is considered an adulteration also.

TABLE 8. COMPOSITION OF ICE CREAM MIXES FOR 10% ICE CREAM ON 1000 LB. BASIS

18% cream	366.1	415.6	210.7*	491.5	385.5	500.8
Evaporated milk	379.7	....	....	....	....	....
Whole Milk	109.2	220.4	416.7	....	311.5	259.2
Plain condensed whole milk	....	219.0	....	....	....	....
Condensed skim milk	....	....	227.6	....	....	....
Sweet condensed whole milk	....	....	....	....	238.0	....
Sweet condensed skim milk	....	....	....	285.0	....	163.9
Sugar	140.0	140.0	140.0	10.3	40.0	71.1
Gelatin	5.0	5.0	5.0	5.0	5.0	5.0
Water	....	....	....	208.2	....	....
Total	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0
Skim milk powder	45.8	110.0	....	....	....	....
Butter	89.6	119.2	68.9	117.8		
Whole milk	719.6	....	1224.0			
Skim milk	....	....	....	1174.0		
Sugar	140.0	140.0	140.0	140.0		
Gelatin	5.0	5.0	5.7	5.7	....	
Water	....	625.8	....	....	....	
Total	1000.0	1000.0	1438.6	1437.5		
Water to be removed by vacuum pan	....	....	438.6	437.5		

These formulas are extracted from the extensive tables given by Mojonnier and Troy.
* Forty per cent cream.

[8] Mojonnier and Troy, "Technical Control of Dairy Products," Mojonnier Bros. (1925).

Fat

Gerber Method—The fat content of ice cream is easily determined by the Gerber method. This method applies equally well to ice cream containing cocoa powder or chocolate flavor. Allow the ice cream to come to room temperature by standing or warming slightly. Ten cc. of sulfuric acid (to 13 parts of water add 87 parts of sulfuric acid sp. gr. 1.82, for all ice cream except chocolate, for which, to 6 parts of water 94 parts of the acid are added) is measured into the ice cream butyrometer, Fig. 48, which is balanced on a suitable balance. Five g. of the properly prepared sample is carefully weighed into the flask. Add from 4.5 to 5.5 cc. of water according to the volume of the butyrometer and 1 cc. of amyl alcohol. Stopper and shake until all of the curd is dissolved and then mix the acid remaining in the neck by inverting several times. Centrifuge for 6 minutes. Remove the butyrometer and read in the same manner as directed under milk.

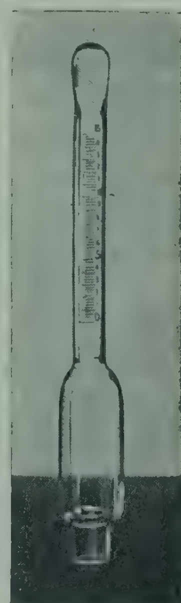

Fig. 48. Gerber Ice Cream Butyrometer

If the ice cream contains nuts or fruit pulp or fruit, it should be squeezed through cheese cloth, or if a very exact analysis is desired, the entire sample may be passed through a food grinder until the nuts and fruits have become incorporated into the ice cream. If the ice cream has separated out, it need not be discarded. It should be warmed to about 35–40° C. and passed through mechanical cow or other type of colloid mixer, which will rehomogenize the ice cream sufficiently so that a correct analysis may be made.

Roese-Gottlieb Method—Transfer about 5 g. of ice cream accurately weighed by difference with the aid of a Mojonnier pipette and a carriage to a Jacobs-Singer separatory flask or a Mojonnier extraction tube, or weigh directly into the lower section of a Jacobs-Singer separatory flask. Add 5–6 cc. of water and 2 cc. of ammonium hydroxide, warm to 60° C., stir thoroughly, and then proceed as in the method described for milk. Care must be taken to add the full amount of alcohol. Divide the weight of the fat found by the exact weight of the sample transferred and multiply by 100 to obtain the percentage fat.

Total solids, proteins, ash, preservatives, coloring matters may all be determined by methods similar to those described in prior sections of

the text. Sugars must be determined by the methods for mixtures detailed in the chapter on sugar. "Stabilizing agents" is just another word for emulsifying agents and thickening agents and these substances may be detected in a manner entirely analogous to the procedures discussed in the sections on cream.

Total Food Solids per Given Volume

The sample must be received in the original container in a frozen condition, or in a container of known volume which has been completely filled with the frozen product. Weigh the ice cream and its container as received in a solid condition to the nearest gram or tenth of a gram. Transfer the ice cream to some other sealed or covered container and clean and dry the original container. Weigh. The weight of the ice cream, plus its container, less the weight of the container, equals the net weight of the ice cream. Determine the total solids of the ice cream by transferring about 5 grams of the ice cream which has been allowed to come to room temperature, accurately weighed with the aid of a Mojonnier pipette and a carriage, to a tared flat-bottomed dish containing sand. Place in the oven to dry. The gain in weight of the dish is the total solids. This gain in weight divided by the weight transferred to the dish and multiplied by 100 is the per cent total solids.

The net weight of the ice cream, multiplied by the per cent total solids, equals the weight of the total food solids in the ice cream. This weight, divided by the volume of the original container, gives the total food solids per given volume. If the volume of the container is accepted as the volume stated on the label, the actual volume of the container need not be determined. If it is desired to obtain the exact volume, it may be done as detailed in the first chapter by measurement. If the stated volume is given in pints, quarts or gallons, the weights which were taken in grams must be multiplied by the factor, 0.0022, in order to convert to pounds. If the original volume was a pint, the ratio should be multiplied by 8/8; if the original volume was a quart, the ratio should be multiplied by 4/4 to bring the expression in the form of lbs./gallon. Any other weight/volume relationship is multiplied by similar factors to arrive at the lbs./gallon expression which is the customary ratio.

These calculations may be summarized as follows:

gross weight in g. − weight of container in g. = net weight in g.
net weight in g. × % total solids = weight of total solids in g.
weight of total solids in g. × 0.0022 = weight of solids in lbs.

$$\text{weight of solids in lbs. divided by original vol.} = \frac{\text{lbs.}}{\text{given volume}}$$

$$\frac{\text{lbs.}}{\text{given volume}} \times \text{appropriate factor} = \frac{\text{lbs.}}{\text{gallon}}$$

Thus, if in a one pint ice cream sample we find:

gross weight	258 g.
container	27 g.
net weight	231 g.
total solids	37.2%

then

$$231 \times 0.372 = 85.9 \text{ g.}$$
$$85.9 \times 0.0022 = 0.189 \text{ lbs.}$$
$$\frac{0.189}{\text{pint}} \times \frac{8}{8} = \frac{1.51 \text{ lbs.}}{8 \text{ pints}} = \frac{1.51 \text{ lbs.}}{\text{gallon}}$$

Overrun [9]

Overrun is the increase in volume due to the amount of air incorporated into the ice cream during the agitation and freezing process. It is generally expressed as percentage overrun. It may be determined by the following procedure. Press an ice cream sampler of exactly 50 cc. volume, a short, wide tube open at both ends, into the frozen ice cream, until it is below the surface. Allow it to remain until chilled and remove by working from side to side while drawing out. Trim off the ice cream protruding from both ends with a sharp knife and wipe the exterior dry. Transfer the ice cream to a beaker, or place the ice cream directly into a funnel resting in a 250 cc. volumetric flask. Transfer the ice cream to the flask with the aid of exactly 200 cc. of warm water and the aforementioned funnel. Add 1 to 2 cc. of ether to destroy the foam and then fill the flask to the 250 cc. mark by adding water from a burette. Record the exact amount of additional water and ether added. The percentage overrun may be calculated by the following series of expressions:

$$50 \text{ cc. (volume of ice cream)} - \text{volume of ether and water} = \text{volume of ice cream before agitation and freezing}$$

$$\frac{\text{volume of ether and water}}{\text{volume of ice cream before agitation and freezing}} = \%\ \text{overrun}$$

[9] Benkendorf, Wisconsin Exptl. Sta. Bull. No. 241 (1914).

For example,

$$50 \text{ cc.} - (22 \text{ cc. water} + 2 \text{ cc. ether}) = 26 \text{ cc.}$$

$$\frac{24 \text{ cc.}}{26 \text{ cc.}} = 92.3\% \text{ overrun}$$

Foreign Fat

To a quart of ice cream add an equal volume of water in a large beaker or Erlenmeyer flask and bring the mixture to a boil. Add 25 cc. of 20 per cent copper sulfate solution and filter hot through a large Büchner funnel with the aid of suction. Drain and dry the precipitate in a vacuum oven. Mix with an equal amount of anhydrous copper sulfate, place in a large thimble and extract with petroleum ether in a Soxhlet extractor as described in Chapter I, or extract with 3 successive portions of petroleum ether. Evaporate off the petroleum ether, dry the fat in a constant temperature oven at 75° C. and proceed to examine the fat as directed in the chapter on fats and oils, Chapter VIII.

Milk Sherbet

In some states another frozen product made from the same ingredients as ice cream but containing only a small percentage of milk solids is allowed to be sold under the name of milk sherbet. In New York State, milk sherbet must not contain more than 5 per cent of milk solids. This food product is analyzed exactly as is ice cream. However, it is more important, or rather, more often necessary to determine the casein content in order to control the maximum limits of milk solids.

Ices

Ices are another type of frozen product but ices are not permitted to contain any milk solids. They are made from sugar and water, flavor, color and stabilizer. They must be rigidly controlled as to milk content for religious, dietary and medicinal reasons, as well as for the prevention of fraud.

Detection of Milk Powder in Foods

The use of skim milk powder and even whole milk powder as a binder or filler in different food products has increased in recent years. Standards have been established to govern this practice by governmental agen-

cies. In some instances their use has been totally prohibited in certain food products as for example in New York State, in ices and ice sherbets, in order to prevent substitution in place of a better product such as milk sherbet or even ice cream. In meat products, their use is sometimes prohibited for dietetic or religious reasons. Milk powder as a binder in sausages, a filler in ices and an anti-crystallizing agent in frozen eggs, are illustrations of the varied use of this product.

Milk has three main characteristic constituents, milk fat, casein, and milk sugar or lactose. The detection of any one of these in a food would be proof of the addition of milk product to that food. In the case of a food containing an oil or fat, the addition of whole milk powder would be evidenced by the alteration in the constants of the fat or oil of that food. Thus, for example, the Reichert-Meissl value would be raised. In food products which contain no fat, the problem of the detection of added milk product resolves itself to one of the identification of casein and lactose. The method for lactose depends upon the complete precipitation of all proteins, split-proteins, gelatin and pseudo-gelatins by tannic acid and neutral lead acetate, identification by means of Fehling's, Barfoed's, Tauber's and mucic acid tests and estimation by the usual methods. The method for casein depends upon its solubility in 3 per cent oxalate solution, whereas other proteins including vitellin are insoluble. The casein is subsequently estimated by the usual methods.

Jacobs Method—*Preparation of sample.* (1) *Meats.* Comminute the meat or other meat product such as sausage and weigh 100 g. into a 600 cc. beaker, add 200 cc. of water, heat to boiling and boil gently for 5 minutes. Stir the contents of the beaker frequently during this and other extractions to prevent bumping. Remove the beaker and cool under cold running water with constant stirring. Place in a refrigerator until the fats have solidified and filter by decantation through a Büchner funnel fitted with a rapid filter paper. Repeat the entire extraction with two 150 cc. portions of water, following each detail. Finally transfer the residue to the filter and press dry. Transfer the filtrate to an evaporating dish or to a beaker and evaporate on a steam bath or a hot plate regulated at low heat to a volume of 25 cc. From this point proceed as detailed in the method.

(2) *Eggs.* Weigh, by difference, approximately 40 g. of the frozen egg yolks or other egg products into a liter volumetric flask containing 600 cc. of water, mix gently, fill to the mark with water and shake gently. Filter through an 18½ cm. fluted filter paper. If the filtrate is cloudy,

allow the filtration to proceed until drops of the filtrate become clear. Return the cloudy filtrate to the filter and wash the receiving flask twice with clear filtrate, returning the washings to the filter. Evaporate 500 cc. of the filtrate on a steam bath and proceed as directed in the method.

(3) *Ices and sherberts.* Use 25 g. and proceed with the method.

(4) *Other food products.* Prepare as directed above a hot water extract, or cold water extract or use the sample unchanged according to the character of the sample.

Method for Lactose. Transfer the 25 cc. prepared as directed above to a 100 cc. volumetric flask with not more than 25 cc. of water. Mix thoroughly. Add 10 cc. of 20 per cent freshly prepared tannic acid solution and shake the flask. Add 5 cc. more. Fill to the mark after allowing to stand for half an hour. Mix gently and centrifuge or allow to stand overnight. Filter through a dry, small filter, discarding the first 15 cc. Transfer a 50 cc. aliquot to a 100 cc. volumetric flask and add 10 cc. of saturated neutral lead acetate solution. Make to volume. Allow to stand or centrifuge. Filter through a small, dry filter, discard the first portion, and delead by precipitating lead as lead sulfate with anhydrous potassium sulfate. Allow to settle. Filter through a small, dry filter and discard the first portion. Test the filtrate for reducing sugars by the Fehling or Benedict test as directed in the chapter on sugars, Chapter IX. If no positive test is obtained, lactose as well as other reducing sugars are not present. If a positive test is obtained, test the filtrate with Barfoed's or Tauber's reagent, as detailed in the chapter on sugars. If no positive test is obtained with these reagents, the reduction in the case of the Fehling test is due to a disaccharide. If the Tauber or Barfoed test is also positive, a reducing monosaccharide is present and the filtrate should be tested for lactose by applying the mucic acid test.

Take 25 cc. of the deleaded filtrate, dilute to 100 cc., add 20 cc. of nitric acid and evaporate to 20 cc. on the steam bath or on a low heat hot plate. Dilute again to 100 cc., add 20 cc. more of nitric acid and evaporate to 20 cc. as before. Allow to stand for 24 hours. Add 10 cc. of water and allow to stand another 24 hours, to permit the mucic acid to crystallize. Pass through a small filter and wash the mucic acid crystals with 30 cc. of water to remove the acid. Return the filter and contents to the original beaker. Add 30 cc. of ammonium carbonate solution (consisting of 1 part ammonium carbonate, 19 parts water and 1 part of ammonium hydroxide) and heat the mixture in a water bath at 80° C. for 15 minutes with constant stirring. Wash the filter paper

and the contents several times with hot water by decantation, passing the washings through a filter paper, to which finally transfer the residue. Evaporate the filtrate to dryness on a water bath, avoid unnecessary heating which causes decomposition, add 5 cc. of nitric acid (sp. gr. 1.15), stir the mixture thoroughly and allow to stand for 30 minutes. Collect the precipitated mucic acid on a weighed Gooch crucible, wash with 10–15 cc. water, then with 60 cc. 95 per cent alcohol and then a number of times with ether. Dry in a constant temperature oven for 3 hours and weigh. Multiply the weight of mucic acid by 1.33 to convert to galactose and then convert to lactose monohydrate by multiplying by 2.

The lactose may be estimated by gravimetric or volumetric reduction methods on a portion of the deleaded filtrate, as detailed in the chapter on sugars. If a monosaccharide is present, as is entirely possible in the case of ices, the methods for mixtures should be used. The above method may be used but it is empirical and requires the factor 1.33 because only 75 per cent of the galactose present is recovered as mucic acid, and hence only part of the lactose is recovered also. The method is best used for qualitative identification because only lactose and galactose of the sugars give the mucic acid test.

Maltose is also a reducing disaccharide hence care must be taken in interpreting the reducing test. It does not yield mucic acid.

Casein. 22.5 grams of the original sample are weighed into a 500 cc. Erlenmeyer flask and the 250 cc. of 3 per cent sodium oxalate solution is added. Boil gently for 5 minutes or allow to stand for 4 hours shaking frequently. Add 5 g. of light magnesium carbonate or 1 g. of Filter-Cel, allow to cool and filter. Take 100 cc. of the filtrate (A), add 2 cc. of glacial acetic acid and dilute to 200 cc. Allow to stand for 1 hour or overnight. Filter, return the filter paper and the precipitate to the beaker in which the acetic acid precipitation was made, add 250 cc. of 3 per cent sodium oxalate solution, boil gently for 5 minutes or allow to stand for 4 hours, shaking frequently, add 5 g. of light magnesium carbonate, allow to cool and filter. Take 100 cc. of this filtrate, add 2 cc. of glacial acetic acid, dilute to 200 cc. and allow to stand. If a dense turbidity is formed, deep enough to obscure a stirring rod held in back of the beaker, which later develops into a curdy white precipitate, casein is present.

To obtain quantitative results, determine nitrogen in an aliquot of the filtrate from the second boiling with sodium oxalate solution and then determine the nitrogen in an aliquot of the filtrate after the addition of acetic acid. The difference in the nitrogen determinations, after cor-

rections for dilution and the aliquots taken, multiplied by 6.38 equals casein. Casein × 1.25 equals milk protein.

Casein may also be estimated by the McDowall and McDowell method as described in the section "formal titration of casein," Chapter VI.

Lactosazone Method.—To detect dried skimmed milk in meat products Kerr[10] recommends the following method. To 25 g. of the finely divided meat in a 250 cc. beaker, add 50 cc. of water. Thoroughly break up the meat with a glass rod and boil the mixture for a few seconds. Filter through a wet filter paper and add to 25 cc. of the filtrate 1 g. of good absorbent charcoal. Mix by shaking, boil for a few seconds, cool thoroughly and shake at intervals for 10 minutes. Filter through a small paper or use a filter pump. When the charcoal has completely drained, transfer it to a porcelain dish containing 10 cc. of water and 1 cc. of glacial acetic acid. This is best done by opening the paper, holding it by the clean half, and moving it about in the liquid. The greater part of the charcoal is thus removed from the paper. Stir the charcoal with a glass rod and transfer the mixture to a boiling tube. Heat to boiling for about 10 seconds and filter the hot solution through a small paper into a test tube containing one-half to 1 g. of solid phenylhydrazine-hydrochloride and 2 g. of solid sodium acetate. Mix thoroughly and filter from any insoluble oily residue. Place the tube in a boiling water bath and leave it there for 45 minutes. Remove the tube and allow it to stand at room temperature for at least one hour and preferably longer. Pipette off a little of the deposit, if any, and examine it on a slide under the microscope.

Lactosazone crystallizes in characteristic clumps with projecting spines ("hedgehog" crystals). Recrystallize by filtering through a small paper, washing with a small amount of water, and then passing about 4 cc. of boiling water through the paper into a clean tube. Boil the filtrate and pass through the paper two or three times, boiling between every filtration. On allowing the solution to stand, typical crystals of the osazone separate out. Filter, dry and take the melting point (200° C.). Care must be taken that the phenylhydrazine-hydrochloride solution does not become too concentrated during the boiling process otherwise lactosazone crystals may not separate out.

This method is based on the principle that the lactose is adsorbed on the charcoal and is subsequently liberated by boiling the charcoal in acetic acid solution. The lactosazone is then formed in sodium acetate

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[10] Kerr, *J. Assoc. Official Agr. Chem.* **19**, 410 (1936).

solution by boiling with phenylhydrazine-hydrochloride and the crystals that are formed are identified under the microscope. This is of course only a qualitative test.

Milk Solids in Bread—This method is based on the determination of lactose and the estimation of butterfat using the Reichert-Meissl number, and applies to skim-milk solids as well as whole-milk solids. It is recommended by Hoffman, Schweitzer and Dalby [11] as a reasonably rapid and practical method for this determination.

Remove the crust of the bread, air-dry the crumb, then grind sufficiently to pass a 20-mesh sieve. Digest 50 grams of the prepared material in 400 cc. of water at about 40° C. for 3 hours, and transfer the mixture to a large centrifuge tube. Centrifuge and decant the liquid portion into a 1 liter volumetric flask. Wash the residue four times using 75 cc. of water each time, and separate solids by centrifuging. Decant after each washing and add the liquid portion to the first extract. Add 35 g. of baker's compressed yeast, suspended in a small amount of water, 0.5 g. of ammonium sulfate, and 0.2 g. of sodium bisulfite, and let stand overnight at room temperature stoppered, but with a vent for the escape of carbon dioxide. The ammonium sulfate is used as a yeast stimulant, and the sodium bisulfite retards bacterial action.

After standing overnight add 20 cc. of copper sulfate solution (regular Fehling's A) and add sufficient sodium hydroxide solution to give a definite blue color and clarify the solution. Make up to volume in the liter flask, shake, and filter through a good quality filter paper. Take 50 cc. of the filtrate and determine lactose, using the Munson and Walker gravimetric method as directed in Chapter IX.

Fifty cc. of the filtrate are equivalent to 2.590 g. of bread after correction for the yeast is made. The fat-free milk solids are calculated from the percentage of lactose found, and average 50 per cent lactose with only slight variations. Therefore twice the percentage of lactose found (after calculation to the dry basis) is equal to the percentage of fat-free solids on the dry basis of the bread.

Extract the fat necessary for the Reichert-Meissl number determination by placing 200–300 g. of finely ground air-dried bread, depending upon the fat content, in a 2-liter flask containing 1000 cc. of water and 30 cc. of hydrochloric acid. Digest the mass by boiling for 1 hour or until it shows good digestion, and add 10 g. of Filter-Cel. Filter through a Büchner funnel containing a filter paper upon which is a thin pad of

[11] Hoffman, Schweitzer and Dalby, *Ind. Eng. Chem., Anal. Ed.* 8, 298 (1936).

Filter-Cel. Apply suction until the mass is fairly dried. Transfer the residue to a beaker, stir with ether, and filter again through Filter-Cel into a dry flask. Evaporate the ether, and if the oil is clear it is ready for the Reichert-Meissl number determination. Use about 5 g. of fat and determine the Reichert-Meissl value as detailed in the Chapter on Oils and Fats, Chapter VIII.

The average oil content of flour on the dry basis is 0.7 per cent. This oil has a Reichert-Meissl value of 1, for which allowance must be made in estimating the amount of butterfat in the bread. If other shortening is present, an allowance must be made for the Reichert-Meissl number of the shortening used. The normal variation in the Reichert-Meissl number of butter fat must also be considered in making the calculation.

The amount of butter fat that would be present if the bread contained whole-milk solids is calculated from the percentage of fat-free milk solids found. The Reichert-Meissl number, after correction for fats other than butter fat present, determines whether the amount of butter fat calculated is actually present. If the Reichert-Meissl number does not indicate any butter fat, skim-milk solids were used in the manufacture of the bread. If the Reichert-Meissl number indicated only part of the butter fat necessary to balance the skim-milk solids in the ratio of skim-milk solids to butter fat in whole-milk solids, then partially skimmed milk was used. The factor 0.4115 multiplied by the percentage of skim-milk or fat-free solids gives the amount of butter fat necessary to balance the skim-milk solids. The Reichert-Meissl number alone without a determination of lactose makes an estimation of the milk solids present quite uncertain.

It may be repeated that the effect of the shortening used in the bread is not to be underestimated and therefore more reliance must be placed on the lactose determination.

REFERENCES

Mojonnier and Troy, "Technical Control of Dairy Products," Mojonnier Bros. (1925).

United States Dept. of Agr. Bull. No. 608, "Varieties of Cheese" (1932).

Eckles, Combs and Macy, "Milk and Milk Products," McGraw-Hill (1936).

Burke, "Practical Dairy Tests," Olsen (1935).

Methods of A. O. A. C. (1935).

Wolf, "The Human Fuel," Chapman and Grimes (1936).

Ling, "Textbook of Dairy Chemistry," Wiley (1930).

Department of Agr. Markets, State of New York, Circ. No. 481 (1934).

Department of Agr. Markets, State of New York, Circ. No. 505 (1937).

CHAPTER VIII

OILS AND FATS

EDIBLE oils and fats are the glycerides of fatty acids, so treated as to be wholesome foods. They are found in the fruits, nuts, seeds and roots of plants and vegetables. In animals, fats are present throughout the entire body but especially so in the adipose tissue and bone marrow.

TABLE 9. MORE IMPORTANT ACIDS OF EDIBLE OILS AND FATS

	Formula	Melting point	Occurrence
Acetic Series	$C_nH_{2n}O_2$		
Butyric	$C_4H_8O_2$	—6.5	butter
Caproic	$C_6H_{12}O_2$		butter, coconut, palm
Caprylic	$C_8H_{16}O_2$	16.5	butter, coconut, palm
Capric	$C_{10}H_{20}O_2$	31.3	butter, coconut, palm
Lauric	$C_{12}H_{22}O_2$	43.6	butter, coconut, palm
Myristic	$C_{14}H_{28}O_2$	53.8	butter, coconut, palm, sesame
Palmitic	$C_{16}H_{32}O_2$	62.6	nearly all oils and fats
Stearic	$C_{18}H_{36}O_2$	69.3	nearly all fats and most oils except olive and corn
Arachidic	$C_{20}H_{40}O_2$	77	peanut, rape and cocoa
Lignoceric	$C_{24}H_{48}O_2$	81	peanut
Oleic Series	$C_nH_{2n-2}O_2$		
Hypogaeic	$C_{16}H_{30}O_2$	33	peanut
Oleic	$C_{18}H_{34}O_2$	14	nearly all oils and fats
Rapic	$C_{18}H_{34}O_2$		rape, mustard
Iso-oleic	$C_{18}H_{34}O_2$	44–45	hydrogenated fats
Erucic	$C_{22}H_{42}O_2$	33–34	rape, mustard
Linoleic Series	$C_nH_{2n-4}O_2$		
Linoleic	$C_{18}H_{32}O_2$	below —18	drying and semi-drying oils
Linolenic Series	$C_nH_{2n-6}O_2$		
Linolenic	$C_{18}H_{30}O_2$		poppy seed, soy bean
Clupanodonic Series	$C_nH_{2n-8}O_2$		
Clupanodonic	$C_{18}H_{28}O_2$		cod liver and other fish oils

Edible fats and oils, chemically, are mixtures of fatty acid esters of the trihydroxy alcohol, glycerol. Table 9 details the most common fatty acids of edible oils and fats.

Fats and oils obtained from various sources differ from one another in their physical and chemical properties because they contain varying amounts of different esters. Some of these esters are solid, some liquid, some volatile, some saturated and some unsaturated compounds. Therefore, each ester influences the physical and chemical properties of an oil or fat in some measure according to the amount of that ester in the fat or oil. These differences are the bases of tests for their identification. The principal esters of fats and oils, stated simply, are stearin, palmitin, butyrin, arachidin, olein and linolein. Stearin, palmitin, butyrin and arachidin are saturated compounds, whereas olein and linolein are unsaturated.

Actually, however, it must be understood that the glycerides of fats and oils are not simple esters where all three hydroxy groups of glycerol have condensed with the same acid radical, but are rather mixed glycerides, in which different acid radicals are attached to the same glycerol molecule.

TABLE 10. COMPONENT FATTY ACIDS OF BUTTER FAT, PER CENT WEIGHT[1]

Butyric Acid	2.6— 3.5
Caproic	1.3— 1.9
Caprylic	0.7— 1.6
Capric	1.8— 3.6
Lauric	3.2— 5.7
Myristic	6.9—11.1
Palmitic	22.8—29.1
Stearic	6.5—12.5
Arachidic (?)	0.6— 0.9
Oleic	31.3—41.3
Linoleic	3.6— 5.1

Edible oils are generally classified as follows:

1) Non-drying vegetable oil of the olive oil type, namely, olive, almond, peach kernel and peanut.

2) Non-drying vegetable oil of rape oil type, namely, rape and mustard seed.

3) Non-drying oil of animal type, namely, lard.

4) Semi-drying vegetable oil, namely, cottonseed, corn, sesame and soya.

[1] Hilditch, *Analyst* 62, 250 (1937)

5) Drying vegetable oil, namely, sunflower, poppy, safflower.

6) Fish and marine animal oil, namely, cod liver, menhaden, whale.

7) Vegetable fat, namely, coconut, cocoa butter, cottonseed stearin, palm.

8) Animal fat, namely, butter fat, beef tallow, lard, mutton tallow.

A drying oil is one which has the property of drying, or of being oxidized, that is becoming thick, viscous and forming a membrane on being exposed to air. The terms semi-drying and non-drying refer to slower drying and lack of ability to dry, in the oils so classified.

The analysis of oils and fats is concerned not as in other foods and food products with percentage composition but rather with the physical and chemical properties which serve as a basis for identification. Mixtures of oils and fats will have properties of the individual oils or fats comprising the mixture. The chemical and physical properties of an oil or fat vary between certain limits and because of the comparatively small variation are called constants. Some of the more important physical constants are specific gravity, refractive index, and melting point. Some of the more important chemical constants are the iodine value, saponification value, Reichert-Meissl value, Polenske value, free fatty acids and unsaponifiable residue. Table 11 contains the numerical values of the constants of the more important edible oils.

It is not necessary to determine every constant for every oil. Thus the purity of olive oil can almost always be easily determined by ascertaining the iodine value, refractive index, saponification value, added color and by performing a series of qualitative tests for added oils foreign to olive oil. Similarly, the purity of butter fat may be learned by determining the Reichert-Meissl, Polenske, Kirschner and saponification values and also the melting point and refractive index. These constants will, in general, disclose whether fat foreign to butter has been added. It would, for example, be superfluous to make a Reichert-Meissl determination for ascertaining the adulteration of olive oil.

One fat and one oil, namely, butterfat and olive oil have assumed a far greater importance in the eyes of the public than any other oil or fat. Butterfat being an animal fat and a milk product is of unquestionable value as a food. Olive oil, on the other hand, has not only been credited with food value, in which it is of no more value than any other edible oil, but has, it is claimed, medicinal and therapeutic powers. Nonetheless, it has a distinct flavor which some consumers prefer. Hence, this oil has in the belief of these people a greater monetary value. Consequently

TABLE 11. CONSTANTS OF OILS AND FATS

Name	Sp. Gr. $\frac{15°C.}{15°C.}$	Saponification Value	Iodine Value	Acid Value	Refractive Index at 25°C.	Unsaponifiable Matter	Reichert-Meissl Value	Hehner Value
Almond	0.911–0.921	183–207	93–103	0.5–3.5	1.4593–1.4646[2]	0.75	0.5	96.0
Beef tallow	0.895	196–200	35–42	0.25	1.449 –1.452[3]		0.2–0.5	95.0–96.0
Butter fat	0.930–0.940	210–230	26–38	0.45–35.4	1.4535–1.4560[2]	0.3–0.5	17.0–34.5	86.5–89.5
Coconut	0.926	253–262	6–10	2.5–10	1.4477–1.4495[2]	0.2	6.6–8.4	82.3–90.5
Cocoa butter	0.950–0.974	192–202	33–42	1.1–1.9	1.4537–1.4580[2]		0.3–1	94–95
Cod liver	0.922–0.931	171–190	135–175	5.6	1.4758–1.4783	0.5–2.7	0.2	95.4
Corn	0.921–0.928	187–193	111–128	1.4–2.0	1.4733–1.477	1.5–2.8	4.3	93–95
Cottonseed	0.920–0.925	191–196	103–115	0.6–0.9	1.4743–1.4752[4]	1.1	0.95	95–96
Cottonseed stearin	0.918–0.923	194–195	89–103					96
Lard oil	0.913–0.916	193–198	62–82	1.5	1.4607[2]	0.6		97
Lard	0.934–0.938	195–203	47–66	0.5–0.8	1.450 –1.454[2]		0.2–0.6	93–95
Menhaden	0.923–0.933	188–193	148–185	5–8	1.4787	0.6–1.4	1.2	
Mutton tallow	0.937–0.953	192–196	32–61	1.7–14	1.4545–1.4582[2]		0.3	95–96
Olive	0.915–0.920	185–196	79–90	0.3–1.0	1.4657–1.4667	0.4–1.0	0.6–1.5	95
Palm	0.921–0.924	196–204	49–59	10	1.4603–1.4639[2]		0.9–1.9	94–97
Peanut	0.917–0.926	186–194	85–100	0.8	1.4620–1.4653[2]	0.5–0.9	0.5	95–96
Poppy seed	0.924–0.926	190–195	128–141	2.5	1.4739–1.4743	0.4	0.6	95–96
Rape	0.913–0.917	168–179	94–105	0.4–1.0	1.4649–1.4659[2]	1.5	0.8	94–96
Sesame	0.921–0.925	188–193	103–117	9.8	1.4704–1.4717	0.9–1.3	1.1	95
Soya	0.924–0.927	189–194	122–134	0.3–1.8	1.4723–1.4756	1.3–1.5	0.5–2.8	93–95
Sunflower	0.924–0.926	188–194	120–136	11.2	1.4659–1.4721[2]	0.3	0.5	95
Tea seed	0.911–0.927	188–196	88–90		1.4707		0.6	
White mustard	0.912–0.916	171–174	94–98	5.4	1.4649			96–97

[2] At 40° C.
[3] At 60° C.
[4] At 15° C.

adulteration with a cheaper oil, is a fraud. The importance of olive oil as an article of commerce is easily seen from the fact that 57,433,000 lbs. of the edible oil alone were imported into the United States in 1935. It is of course true, that olive oil is obtained pure directly from its precursor, the olive, just as butter fat may be obtained pure directly from its precursor, milk or cream. Other oils and fats must be refined before being acceptable for food use, for example, cottonseed oil.

Specific Gravity

The specific gravity of oils and fats may be determined by the methods described in Chapter I. The tables of specific gravity of oils are generally given at 15.5° C. Many fats are not liquid at this temperature and hence the specific gravity must be determined at a higher temperature and this figure must then be corrected for expansion. This may be done as follows.

$$\text{sp. gr.} \frac{t^\circ \text{ C.}}{15.5^\circ \text{ C.}} = \frac{W_1}{W}$$

$$\text{sp. gr.} \frac{15.5^\circ \text{ C.}}{15.5^\circ \text{ C.}} = \frac{W_1}{W} + 0.00064 t_1^\circ$$

For all temperatures above 15.5° C.

W_1 = weight of oil
W = weight of an equal volume of water
t° = temperature at which oil is weighed
$t_1^\circ = t^\circ - 15.5^\circ$

0.00064 is an average coefficient of expansion for all oils. The A. O. A. C. directs that the apparent specific gravity be determined at 25° C. or referred to that temperature and it gives the correction factor as 0.0007 for specific gravity determined at any other temperature.

Refractive Index

The refractive index of oils is generally taken at 25° C. and of fats at 40° C. This may be done as described in Chapter II. The correction to be applied for readings taken at a temperature greater than 40° C. is + 0.00038 times the number of degrees over 40° C. A corresponding correction may be applied for readings taken over 25° C. by using the same correction factor.

Melting Point

A very small portion of fat is introduced into a capillary tube by drawing up the melted fat into the tube by suction. The capillary is then placed on ice for at least 30 minutes and preferably overnight. It is removed and attached to a thermometer graduated to 0.2° by means of rubber bands. The thermometer is suspended in a beaker containing water and the point of incipient fusion at which the fat is translucent, and the point of complete fusion at which the fat is transparent, is determined in the usual way.

Titer Test

It is difficult to obtain the solidifying point of an oil or fat. This is much more easily performed on the fatty acids derived from the oil or fat. The procedure followed in obtaining the fatty acids and then determining the solidifying point of these mixed fatty acids is termed the titer test. This constant is more characteristic than that of the melting point. However, the labor and time involved in the method is not commensurate with the value of the result obtained. The reader is referred to one of the texts given in the bibliography at the end of the chapter.

Physical Aspects

The viscosity, surface tension, optical rotation, fluorescence and absorption spectra of oils and fats may be obtained as detailed in Chapter II. These determinations are being made with increasing frequency and may become important means of determining the purity of an oil or fat.

Virgin olive oil gives a yellow fluorescence, probably due to carotene. The oxidation induced by long exposure to air causes a change from yellow to bluish-green or violet tones. A violet fluorescence is particularly noticeable in most oils, as for example, cottonseed, soya, palm kernel, rape seed, peanut, etc., which have been refined under pressure or obtained by solvent extraction, the yellow color that is normally present, probably being destroyed by the processing. The addition of annatto or carotene causes the reappearance of the yellow fluorescence.

Butter and margarine both fluoresce, butter with a yellowish color and margarine with a strong blue. Stadler [5] recommends that the fat be dissolved in petroleum ether, in which solvent a 15 per cent adulteration

[5] Stadler, *Z. Untersuch. Lebensm.* 55, 404 (1928).

of butter with margarine can be detected by the strong bluish fluorescence. Fat separated from artificial cream appears pure white with a bluish fluorescence, whereas genuine cream yields fat which gives a striking yellow color.

Cocoa butter does not have much fluorescence unless refined or purified by a solvent extraction process.

Iodine Value

The unsaturated glycerides of an oil or fat have the ability to absorb a definite amount of iodine, especially when aided by a carrier such as iodine chloride or iodine bromide and thus form saturated compounds. The quantity of iodine absorbed is a measure of the unsaturation of an oil or fat. The iodine value is generally expressed as the number of grams of iodine absorbed by 100 grams of the oil. The two methods usually employed for the estimation of the iodine value are the Hanus method using iodine bromide as the carrier and the Wijs method using iodine chloride as the carrier. The preparation of iodine bromide solution is easier than the Wijs reagent. There is some difference in the iodine values obtained by these two methods but the difference is not greater than the variation in the iodine values of the oils or fats themselves.

Hanus Method—The iodine bromide reagent is prepared by dissolving 13.2 g. of iodine in 1 liter of glacial acetic acid. Add small portions of warmed glacial acetic acid to the iodine. When the iodine is completely dissolved and the mixture is cool add enough bromine to double the halogen content, usually about 3 cc. is sufficient. Dissolve the iodine in one portion of the liter of glacial acetic acid and the bromine in another smaller portion. Estimate the strength of each solution by titrating a small portion with potassium iodide solution and standard thiosulfate. With the aid of these results the exact amount of the bromine solution to be added to the iodine solution may be calculated.

Weigh about 0.1 to 0.5 g. of the oil or fat, according to the unsaturation, into a small capsule. Record the exact weight. Transfer the capsule and its contents to a 250 cc. glass stoppered bottle. Add 10 cc. of chloroform to dissolve the sample and then 25 cc. of the Hanus iodine solution and allow to stand for 30 minutes with occasional shaking. A large excess of iodine should always be present at least 60 per cent. At the end of this period, add 10 cc. of 15 per cent potassium iodide solution, shake thoroughly and then wash down the sides of the bottle and the stopper

with 100 cc. of freshly boiled and cooled water. Titrate with the standard 0.1 *N* sodium thiosulfate solution, adding it with constant shaking until the yellow color of the iodine has almost disappeared. Add 2 cc. of a 1 per cent starch solution to act as an indicator and continue the titration with the thiosulfate. When the blue color has almost disappeared, stopper the bottle and shake vigorously so that any iodine remaining in the chloroform layer will pass into the potassium iodide solution. Complete the titration. Run two blank determinations on an equal portion of Hanus solution. The number of cc. of 0.1 *N* sodium thiosulfate solution required by the blank, less the quantity used in the determination gives the thiosulfate equivalent of the iodine absorbed by the oil or fat. Calculate the grams of iodine absorbed by 100 g. of the fat or oil. This is the Hanus iodine value.

Wijs Method—The iodine chloride solution is prepared by dissolving 13 g. of iodine in 1 liter of glacial acetic acid. Then pass in sufficient washed and dried chlorine until the halogen content is almost but not quite doubled. This may be ascertained by thiosulfate titrations. An excess of iodine is permissible but an excess of chlorine must be avoided.

Weigh accurately from 0.1 to 0.5 g. of the oil or fat, according to the unsaturation of the oil or fat, into a small capsule and transfer the capsule and its contents to a 250 cc. glass stoppered bottle. Add 15 cc. of chloroform or carbon tetrachloride to dissolve the oil or fat and then 25 cc. of the Wijs iodine solution, allowing the pipette used to deliver the iodine solution to drain for a definite time. Place the bottle in a dark place and allow to stand for 30 minutes. At the end of this period, add 20 cc. of 15 per cent potassium iodide solution, stopper the bottle and shake thoroughly, and wash down the sides of the bottle and the stopper with 100 cc. of recently boiled and cooled water. Titrate with standard 0.1 *N* sodium thiosulfate solution, adding the reagent with constant shaking until the yellow color of the iodine has almost disappeared. Add 2 cc. of a 1 per cent starch solution and continue the titration. When the blue color has almost disappeared, stopper the bottle and shake vigorously so that any iodine remaining in the organic solvent layer will pass into the water layer. Complete the titration. Run two blank determinations on an equal portion of the Wijs reagent, allowing the pipette to drain for the same length of time as for the unknown. The number of cc. of 0.1 *N* sodium thiosulfate solution required by the blank less the quantity used in the determination gives the thiosulfate equivalent of the iodine

absorbed by the oil or fat. Calculate the grams of iodine absorbed by 100 g. of the fat or oil. This is the Wijs iodine value.

Thiocyanogen Value

Thiocyanogen, $(CNS)_2$ may be regarded as a pseudo-halogen in a manner analogous to ammonium being regarded as a pseudo-alkali metal. It may be obtained by the action of bromine on lead thiocyanate in ethereal solution. It forms colorless crystals melting at −3° to −2° C., and reacts as a typical halogen hence it will be absorbed by unsaturated glycerides as is iodine. By means of the thiocyanogen method and a series of mathematical formulae derived from the theoretical thiocyanogen and iodine values, the percentage of oleic, linoleic and saturated acids in an oil or fat may be estimated, provided other unsaturated acids are not present in large amounts.

Preparation of Lead Thiocyanate: Dissolve 250 g. of the finest C. P. neutral lead acetate $[Pb(CH_3COO)_2.3H_2O]$, in 500 cc of water. Dissolve likewise 250 g. of C. P. potassium thiocyanate in 500 cc. of water. Add the lead acetate solution to the potassium thiocyanate solution slowly and with stirring. Filter off the precipitated lead thiocyanate on a Büchner funnel and wash successively with water, alcohol, and ether. Dry the lead thiocyanate as much as possible by drawing air through it. Remove from the funnel and dry on a watch glass in a phosphorus pentoxide desiccator for 8 to 10 days before using. This lead thiocyanate should be a greenish or yellowish white crystalline material; if it is at all discolored it must be discarded. Precipitated lead thiocyanate may be kept for a period not exceeding two months.

Preparation of Acetic Acid: Acetic acid is conveniently dehydrated by refluxing with acetic anhydride. In a 3 liter Florence flask, with a large test tube set in the neck and through which cold water is passed to serve as a condenser are placed 2 liters of C. P. glacial acetic acid (99.5 to 100.0 per cent) and 100 cc. of acetic anhydride (90 to 100 per cent). This mixture is refluxed over an oil bath for 3 hours at approximately 135° C. After the anhydrous acid has cooled to room temperature, it should be placed in a cleaned and dried glass-stoppered bottle.

Preparation of an 0.2 N Solution of Thiocyanogen: For the preparation of 1 liter of solution, suspend 50 g. of the dry lead thiocyanate in 500 cc. of anhydrous acetic acid; dissolve 5.1 cc. of C. P. bromine in another 500 cc. of acid. Two glass-stoppered acid bottles of 2 or 3 liter capacity, which have previously been cleaned and dried, should be used for this

purpose. Add the bromine solution to the lead thiocyanate suspension slowly, in small portions, and shake vigorously, between each addition, until the solution is completely decolorized. After all the bromine has been added, allow the precipitated lead bromide and the excess lead thiocyanate to settle out, then filter the solution as rapidly as possible. A 13 cm. Büchner funnel and qualitative filter paper together with two 2-liter pressure flasks are used for the filtration. They are previously dried for 1 hour at 105° C. The entire solution is filtered by suction into the one flask, when the funnel, containing the paper and some cake, is transferred to the second flask and the solution refiltered. It should be perfectly clear upon the second filtration. The solution should be stored in glass-stoppered brown bottles and kept in a cool place, 15.5–21° C. (60–70° F.). If it is convenient, the following method for the preparation of the thiocyanogen solution can be used to advantage. Suspend 50 g. of the dry lead thiocyanate in 600 cc. of anhydrous acetic acid in a round-bottomed 2-liter flask, equipped with a mechanical stirrer and dropping tube. Slowly add with agitation 5.1 cc. of bromine suspended in 200 cc. of the dry acid in the dropping tube. The acetic acid-bromine solution should be added at a rate such that the liquid in the reaction flask remains only faintly tinged with brown. When the entire bromine-acetic acid solution has been added, the dropping tube is rinsed out with an additional 200 cc. of the dry acid which is added immediately to the reaction mixture. When the bromine has all reacted, as indicated by the color of the reaction mixture, the agitation is ceased, the precipitated lead bromide allowed to settle, and the thiocyanogen solution filtered as described above.

Determination of the Thiocyanogen Number: Weigh 0.1 to 0.3 g. of oil accurately into a dry 125 cc. glass-stoppered flask. Add from a pipette 25 cc. of thiocyanogen solution and allow to stand for 24 hours in the dark. The storage place should be from 18–21° C. (65–70° F.) in temperature and should not exceed 21° C. for any length of time. The size of the sample is governed largely by the expected thiocyanogen absorption. The excess thiocyanogen should be at least 100 per cent and preferably 150 per cent of the amount absorbed by the oil, although a greater excess seems to do no harm. At the end of 24 hours, 1 g. of dry powdered potassium iodide is added to the flask and the flask is swirled rapidly for 2 minutes. It is advisable to agitate the blank determination for 3 minutes. Mechanical agitation such as is employed at times for iodine values is found very satisfactory for thiocyanogen values. Then add 30 cc. of water and titrate the liberated iodine with

0.1 N sodium thiosulfate solution, using starch as an indicator. At least three blanks should be run with the samples. The solution should also be titrated at the beginning of the 24-hour period. If the drop is more than 0.2 cc. on the blank titrations, the solution is decomposing too rapidly and erratic and low figures will be the result.

An iodine value must be determined by the regular Wijs method as a factor in the calculation.

$$TV = \frac{(\text{blank titration} - \text{titration of sample}) \times (\text{N of } Na_2S_2O_3) \times 12.69}{\text{weight of sample}}$$

in which

TV = thiocyanogen value of the oil.
N = normality.

or the calculation may be expressed as follows:

$$TV = \frac{(\text{blank titr.} - \text{titr. of sample}) \times Na_2S_2O_3 \text{ factor } (IV) \times 100}{\text{weight of sample}}$$

in which the $Na_2S_2O_3$ factor (IV) is expressed as grams of I_2 per cc.

TV = thiocyanogen value of the oil.
IV = iodine value of oil Wijs method.

This new index makes it possible to calculate the composition of oil composed of the glycerides of oleic, linoleic and a group of saturated acids. Hence the method may, in the main, be applied to most of the common edible oils. From the theoretical values of the thiocyanogen and iodine values of the glycerides the following equations may be set up.

	Theoretical Iodine Value	Thiocyanogen Value
Linoleic glycerides (L)	173.3	86.6
Oleic glycerides (O)	86.0	86.0
Saturated glycerides (S)	0	0

then placing

$$L + O + S = 100$$
$$173.3L + 86.0\ O + S = IV \times 100$$
$$86.6L + 86.0\ O + S = TV \times 100$$

we have a set of simultaneous equations that may be solved.

Calculation of Fat Composition: The following calculations may be used when the iodine number and thiocyanogen number are determined on the fat directly and it is desired to express the percentages of the various acids as glycerides. In these formulas the derivation of which is shown above, no unsaturation greater than linoleic is assumed to be present:

$$L = 1.154\ (IV - TV).$$
$$O = 1.162\ (2TV - IV).$$
$$S = 100\% - (L + O).$$

in which

IV = iodine number of the oil.
TV = thiocyanogen number of the oil.
L = per cent of linoleic glycerides.
O = per cent of oleic glycerides.
S = per cent of saturated glycerides.

When the iodine number and thiocyanogen number have been determined directly or indirectly on the free fatty acids of the oil, the percentages of the various acids may be determined, not as glycerides but as per cent of acid, in the following manner:

$$\%\ LA = 1.104\ (IV - TV)$$
$$\%\ OA = 1.112\ (2TV - IV)$$
$$\%\ SA = 100\% - \%\ (LA + OA)$$

All glassware and chemicals used in the preparation or handling of thiocyanogen solutions must be absolutely free from water. The glassware should be scrupulously cleaned with cleaning solution, water, alcohol, and ether and then dried for 1–2 hours in an oven at 105° C. The thiocyanogen solution should not be exposed to air, heat, or light for any length of time. The 0.2 *N* thiocyanogen solution cannot be used after its decomposition exceeds 0.2 cc. of 0.1 *N* sodium thiosulfate solution for 25 cc. over a period of 24 hours. This rate of decomposition should not be exceeded in less than 7 days.

Saponification Value

Saponification is the hydrolysis of esters. This is generally done by boiling the esters with alkali. Oils and fats are the fatty acid esters of the trihydroxy alcohol, glycerol. The saponification value or Koettstorfer value is the number of milligrams of potassium hydroxide required to

saponify 1 gram of oil or fat. Since 1 gram of an oil or fat containing glycerides of the low molecular weight fatty acids will have more molecules than an oil or fat containing glycerides of the high molecular weight, the number of milligrams of potassium hydroxide required to saponify the oil or fat will be greater in the former than in the latter case. Thus the saponification value of butter is higher than that of beef tallow because the former contains the low weight fatty acid, butyric acid.

Purify a portion of alcohol by refluxing 1.2 liters of 95 per cent alcohol over 10 g. of potassium hydroxide and 6 g. of aluminium foil for about 30 minutes. Distill by heating on a steam bath, discard the first 50 cc. of the distillate. Dissolve 40 g. of potassium hydroxide in one liter of the distillate and keep the solution in a glass stoppered bottle in the dark.

Weigh accurately 5 g. of the oil or fat into a 300 cc. Erlenmeyer flask. This may be done with the aid of a Mojonnier pipette and carriage, by difference, or by some other suitable means. Add 50 cc. of the alcoholic potassium hydroxide with a pipette, allowing the pipette to drain for a definite time. Connect the flask with an air condenser and reflux until the fat is completely saponified, which point is reached when a solution free of fat globules is obtained. Cool, titrate the excess potassium hydroxide with 0.5 *N* hydrochloric acid using phenolphthalein as indicator. Run a blank along with the sample, using the same 50 cc. pipette and allowing the pipette to drain for the same length of time. Subtract the number of cc. of 0.5 *N* hydrochloric acid used in the determination from that used in the blank. The remainder is the number of cc. of 0.5 *N* hydrochloric acid equivalent to the potassium hydroxide used to saponify the oil or fat. Calculate the number of milligrams used for 1 gram of oil or fat. This number is the saponification value.

To calculate the saponification value, the following formula may be used.

$$\text{Saponification value} = \frac{(a - b) \times 28.05}{\text{weight of oil}}$$

where

$a =$ the number of cc. of 0.5 *N* hydrochloric acid required for the blank,

and

$b =$ the number of cc. of 0.5 *N* hydrochloric acid required for the determination.

Hehner Value

Most of the fatty acids of an oil or fat are insoluble in water. In the case of those fatty bodies having fatty acids of low molecular weight which are more soluble in water, such as butter, coconut and palm kernel oils, the percentage of insoluble acids will be lower. The Hehner value is a number expressing the percentage of insoluble fatty acids plus unsaponifiable matter in an oil or fat.

The alcohol in the residue from the saponification value determination is evaporated off and the soaps are completely dissolved in sufficient warm water and transferred to a beaker. Ten cc. of hydrochloric acid, sp. gr. 1.12, is added and the mixture heated until all the fatty acids rise to the top as an oil. Cool and place the beaker in a refrigerator. Weigh a thick sheet of filter paper. Wet it thoroughly and place it in a Büchner funnel. Filter the liquid and transfer the cake of fatty acids to the filter. Wash out all the fatty acids from the beaker by using hot water and freezing the oil formed before filtering. Wash the fatty acids on the filter thoroughly with cold water. Cool the funnel. Transfer the filter and fatty acids to a weighed beaker and dry at 100 to 105° C. Cool and weigh. Calculate the percentage of insoluble fatty acids. This is the Hehner value.

Reichert-Meissl and Polenske Values

Some of the fatty acids obtained from fats and oils are volatile with steam and some are not. The volatile fatty acids consist mainly of butyric, caproic, caprylic, capric, lauric and myristic acid. Butyric and caproic which are water soluble and caprylic and capric which are slightly water soluble are the acids estimated in the Reichert-Meissl value. Capric, lauric and myristic comprise the acids estimated in the Polenske value. There is no definite line of demarcation, for the longer the steam distillation is carried on, the more volatile fatty acid is obtained. Nonetheless, if the distillation is carried out under rigidly controlled conditions, consistent results can be obtained. In these methods, the fat or oil is saponified with glycerol soda solution, the fatty acids are liberated from the soaps formed by the addition of acid and are subsequently isolated by distillation.

The Reichert-Meissl value is the number of cc. of 0.1 N sodium hydroxide required to neutralize the water soluble fatty acids distilled from 5 g. of the fat or oil under the specific conditions of the method. The Polenske value is the number of cc. of 0.1 N sodium hydroxide required to neutralize the water insoluble but alcohol soluble fatty acids

distilled from 5 g. of the fat or oil under the specific conditions of the method.

The A. O. A. C. gives the following conditions for conducting the determination of the Reichert-Meissl and Polenske values. Weigh accurately 5 g. of the sample to be tested into a clean, dry 300 cc. flask; add 20 cc. of the glycerol-soda solution, add 20 cc. of (1:1) sodium hydroxide solution to 180 cc. of pure concentrated glycerol. Heat over a flame or asbestos plate until complete saponification occurs, as shown by the mixture becoming perfectly clear. If foaming occurs, shake the flask gently. Add 135 cc. of recently boiled water drop by drop at first to prevent foaming, then add 6 cc. of sulfuric acid (1:4) and a few fragments of pumice stone, previously heated to a white heat, plunged into water and kept there until used. Distill, without previously melting, the fatty acids, using an apparatus of the approximate dimensions illustrated in Fig. 49. Rest the flask on a piece of asbestos board having a hole 5 cm. in diameter in the center, and so regulate the flame as to collect 110 cc. of the distillate in as near 30 minutes as possible and to allow the distillate to drip into the receiving flask at a temperature not higher than 18°–20° C.

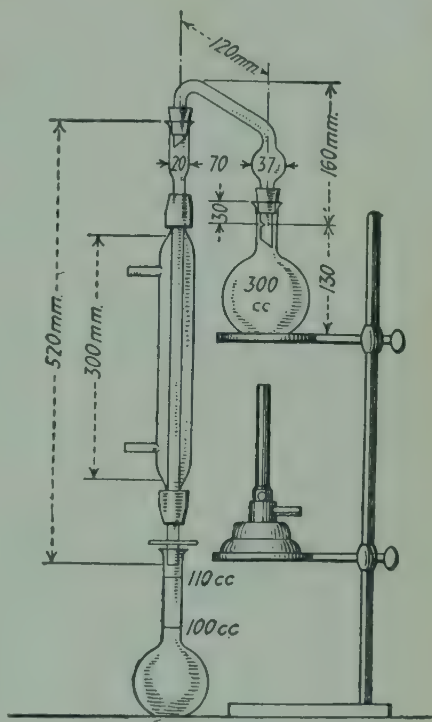

FIG. 49. Reichert-Meissl Polenske Apparatus

(From Thurston's Pharmaceutical and Food Analysis. D. Van Nostrand Co., Inc.)

When the distillation is complete, substitute for the receiving flask a 25 cc. cylinder to collect any drops that may fall after the flame has been removed. Mix without violent shaking, immerse the flask containing the distillate almost completely in water at 15° C. for 15 minutes, filter the 110 cc. of distillate through a dry filter paper 9 cm. in diameter, and titrate 100 cc. with the standard sodium hydroxide solution, using phenolphthalein indicator. The pink color should remain unchanged for 2 or 3 minutes. The Reichert-Meissl value is the number of cc. of 0.1 N sodium hydroxide solution used times 1.1, after this result is corrected for the figure obtained in a blank determination.

Remove the remainder of the soluble acids from the insoluble acids upon the filter paper by washing with 3 successive 15 cc. portions of water, previously passed through the condenser, the 25 cc. cylinder and the 110 cc. receiving flask. Then dissolve the insoluble acids by passing 3 successive 15 cc. portions of neutral alcohol, 95 per cent by volume, through the filter paper, each portion having previously passed through the condenser, the 25 cc. cylinder and the 110 cc. receiving flask. Titrate the combined alcoholic washings with the standard sodium hydroxide solution, using the phenolphthalein as indicator. The Polenske value equals the number of cc. of alkali solution required for the titration.

Kirschner Value

The silver salt of butyric acid is soluble in water. By use of this fact the butyric acid portion of the volatile fatty acids may be separated and estimated. The Kirschner value is a number dependent on the amount of soluble silver fatty acids in the Reichert-Meissl distillate. Butter fat gives Kirschner values from 19 to 26; coconut oil gives an average value of 1.9; palm kernel oil gives an average value of 1.0; the majority of other oils and fats give values varying from 0.1 to 0.2.

To 100 cc. of the Reichert-Meissl distillate, in a 200 cc. Erlenmeyer flask, add 6 drops of phenolphthalein indicator and titrate to a faint pink with 0.1 N barium hydroxide solution. Add 0.3 g. of finely powdered silver sulfate. Allow the mixture to stand for an hour with frequent shaking during that period. Filter and transfer 100 cc. of the filtrate to a 300 cc. flask. Add 10 cc. of sulfuric acid (1 : 40), 35 cc. of water and several small pieces of pumice stone. Distill 110 cc. in about 20 minutes, using the Polenske apparatus, Fig. 49. Titrate 100 cc. of the distillate with a 0.1 N barium hydroxide solution. Make a blank determination. The Kirschner value may be calculated by use of the following formula:

$$K = \frac{A \times 121\ (100 + B)}{10{,}000}$$

where

K = the Kirschner Value.

A = the number of cc. of 0.1 N $Ba(OH)_2$ used to neutralize the distillate from the soluble silver salts of the fatty acids (second distillate) less the blank.

B = the number of cc. of 0.1 N $Ba(OH)_2$ used to neutralize the 100 cc. of Reichert-Meissl distillate (first distillate).

Saturated and Unsaturated Fatty Acids

The lead salts of the solid saturated fatty acids, stearic and palmitic, are insoluble in ether. The lead salts of the liquid unsaturated fatty acids, oleic, linolic, linolinic, are soluble in ether. The lead-salt-ether method is based on this difference in solubility. The solid unsaturated fatty acids, erucic, iso-oleic, etc., are somewhat soluble in ether, although with difficulty. The liquid saturated acids and the saturated acids having a moleclular weight lower than myristic give lead salts that are ether soluble. Hence the lead-salt-ether method cannot be applied to fats and oils that contain erucic, elaeostearic, chaulmoogric, hydnocarpic or similar acids, nor to hydrogenated materials that contain iso-oleic acid, nor to coconut or palm kernel oils that have low molecular weight fatty acids that give ether soluble lead salts. This method is of limited value, time consuming and laborious, therefore the details are not given.

Free Fatty Acids or Acid Value

Oils and fats contain more or less fatty acids according to the conditions of manufacture, age and storage. The glycerides are hydrolyzed to a small degree by enzymes, air and possibly bacteria. The increase in free fatty acids is generally accompanied by a rancid odor although the odor itself is not due to the acidity. The acid value is the number of mg. of potassium hydroxide required to neutralize the free fatty acids in 1 g. of the oil or fat.

Transfer a weighed quantity of oil or fat, about 20 g., and add 50 cc. of neutral 95 per cent alcohol. Heat to boiling and shake the flask thoroughly to dissolve the free fatty acids. Titrate with aqueous 0.1 N potassium hydroxide, shaking thoroughly during the titration until the pink color persists. Calculate the number of mg. of potassium hydroxide required to neutralize the free fatty acids in 1 g. of the oil or fat from the titration. This is the acid value.

Cholesterol and Sitosterol

All fats and oils contain 0.1 to 2 per cent of aromatic alcohols known as sterols. The sterols are phenanthrene derivatives, are monohydric alcohols and are closely related to the bile acids. Cholesterol is the sterol

characteristic of animal fats and oils and sitosterol is the sterol characteristic of vegetable oils and fats. Adulteration of animal fats, e. g. butter, with vegetable fat like coconut oil may be demonstrated by showing the presence of sitosterol. Similarly, the presence of menhaden or other fish oil in an oil of vegetable origin as linseed can be detected by the presence of cholesterol. One of the methods for this identification, developed by Windaus [6] is based on the precipitation of the sterols with 1 per cent digitonin in alcohol and the subsequent formation of cholesterol and sitosterol acetates. Cholesterol acetate melts at about 114° C. and sitosterol acetate at about 125 to 137° C. Sitosterol is not a pure compound as the older literature reports. Anderson [7] has shown that sitosterol from corn oil is a mixture containing 3 isomeric sterols which differ in their physical properties. The melting point of the sitosterol obtained varies therefore, according to the purity of separation. Mixtures of cholesterol and sitosterol acetate will melt in between these temperatures.

To 50 g. of oil or fat in a separatory funnel, add 20 cc. of a 1 per cent solution of digitonin in 95 per cent alcohol and shake the mixture vigorously for about 15 minutes. Allow the mixture to stand for a time until the emulsion separates. Draw off the lower clear fat layer, being careful not to withdraw any of the bulky, flocculent precipitate which is present in the alcohol layer. Add 100 cc. of ether to the alcohol layer and filter the mixture. Dry the precipitate in air and wash it free from fat with ether. Transfer the precipitate to a tall form 100 cc. beaker and add 2–3 cc. of acetic anhydride. Cover the beaker with a watch glass and boil the mixture gently over a low flame for 30 minutes. Cool and add 30–35 cc. of alcohol, 60 per cent by volume. Stir the contents of the beaker thoroughly. Filter the alcohol solution. Wash the precipitate with more 60 per cent alcohol. Dissolve the precipitate on the filter with hot 80 per cent alcohol and place the filtrate in a cool place, 10° C. or below. After the acetates have crystallized, filter them off and recrystallize them from absolute alcohol. When the crystals are dry, determine the melting point. If the melting point is about 114° C. which is confirmed by the melting point of a second recrystallization of the crystals, only cholesterol acetate is present. On the other hand if the crystals melt between the aforementioned temperature and 125° C., which again is confirmed by the melting point of a second recrystallization, then a mixture of sitosterol and cholesterol acetates is present.

[6] Windaus, *Chem. Ztg.* **37**, 1001 (1913).

[7] Anderson, *J. Am. Chem. Soc.* **48**, 2976 (1926).

Unsaponifiable Matter

Those substances not soluble in water after saponification of fatty bodies are classed under the general term unsaponifiable matter. The substances composing the greater part of the unsaponifiable matter in pure oils and fats are cholesterol and sitosterol. If the unsaponifiable matter exceeds 2 per cent some type of foreign matter is probably present and adulteration of the fat or oil is indicated. The foreign matter may consist of a mineral or similar hydrocarbon oil, wax or fat, spermaceti, or rosin oil. Estimation of the percentage of unsaponifiable matter will definitely establish such types of adulteration.

The Fat Analysis Committee of the Division of Industrial Chemists and Engineers, Am. Chem. Soc., gives the following directions for the determination of unsaponifiable matter. Redistill petroleum ether below 75° C. and make a blank determination by evaporating 350 cc. of the reagent with about 0.25 g. of stearin or other hard fat. The blank must not exceed a few mg. Weigh 5 g. (±0.020 g.) of the prepared sample into a 200 cc. Erlenmeyer flask, add 30 cc. of redistilled 95 per cent alcohol and 5 cc. of 50 per cent aqueous potassium hydroxide. Boil the mixture for 1 hour under a reflux condenser. Transfer to the extraction cylinder (glass stoppered, graduated at 40 cc., 80 cc., and 130 cc. with a diameter of about 1⅜ inches and about 12 inches in height) and wash to the 40 cc. mark with redistilled 95 per cent alcohol. Complete the transfer, first with warm, then with cold water, until the total volume is 80 cc. Rinse the flask with 50 cc. of petroleum ether and add the rinsings to the contents of the cylinder previously cooled to room temperature. Shake as vigorously as possible for 1 minute and allow to settle until both layers are clear, when the volume of the upper layer should be about 40 cc. Draw off the petroleum ether layer as closely as possible by means of a slender glass siphon into a separatory funnel of 500 cc. capacity. Repeat the extraction at least 6 times more, using 50 cc. of petroleum ether for each extraction. Wash the combined extracts in the separatory funnel 3 times with 25 cc. portions of 10 per cent alcohol by volume, shaking vigorously each time. Transfer the petroleum ether layer to a weighed Erlenmeyer flask and distill; or, if desired, evaporate the petroleum ether on a steam bath in a current of air. Heat the flask with the residue until a constant weight is obtained in an oven at a uniform temperature, not less than 100° nor more than 110° C. Deduct the blank from the weight before calculating unsaponifiable matter. Test the final residue for solubility in 50 cc. of petroleum ether at room temperature.

Filter, and wash free from the insoluble residue, if any. Evaporate and dry as detailed above.

Ethyl Ether Method[8]—The oil or fat is saponified by boiling with alcoholic potassium hydroxide solution, and the resulting soap solution is diluted with water and extracted with ethyl ether. The ethereal solution is washed with water, aqueous potassium hydroxide solution and then finally with water. To ensure uniformity, emphasis is laid upon the necessity for attention to detail at every stage.

Weigh accurately a quantity of the oil or fat not exceeding 2.5 g., but not less than 2.0 g. and saponify by boiling for one hour, with occasional swirling, under a reflux condenser with 25 cc. of approximately, but not less than 0.5 *N* alcoholic potassium hydroxide solution.

After saponification, during which no loss of alcohol should occur, transfer the alcoholic soap solution to a separatory funnel, washing in with 50 cc. of water in all.

Extract the soap solution while still just warm, successively three times with 50 cc. of ethyl ether. Use the first quantity of ethyl ether to wash out the saponification flask before adding to the soap solution in the separatory funnel.

Make each extraction by shaking the separatory funnel vigorously, allowing the two layers to separate and clarify, running off the aqueous layer at the bottom of the separatory funnel, and pouring the ethereal solution from the top of the separatory funnel into another separatory funnel containing 20 cc. of water. If the ethereal extracts contain solid suspended matter, pass them through a dry fat free filter into the second separatory funnel, washing the filter subsequently with ethyl ether.

The method can be greatly simplified by the use of a Jacobs-Singer separatory flask. Weigh the oil directly into the lower section. Add the 25 cc. of 0.5 *N* alcoholic potassium hydroxide solution. Attach the section to a reflux condenser through the standard taper joint and saponify for the requisite time. Stopper the lower section with the upper section and add water to the connecting joint. Mix and then extract with 3 successive portions of ethyl ether. Pour off each supernatant ether layer through the mouth of the upper section into a separatory funnel and proceed with the method.

If, for any reason, the presence of metallic soap in the original sample

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[8] Report of Sub-committee on Determination of Unsaponifiable Matter in Oils and Fats, *Analyst* **58**, 203 (1933).

is known or suspected, pour the three ethereal extracts into a second and empty separatory funnel, add 5 drops of hydrochloric acid, and shake vigorously. Wash the combined extracts successively with two quantities of 20 cc. of water, employing vigorous shaking on each occasion, and continue the process beginning with "After one or other of these preliminary treatments, . . ." as detailed below.

In the presence of metallic soaps in the original sample is not known or suspected, rotate the extracts gently without violent shaking with 20 cc. of water and, after allowing to separate, run off the wash water. Then wash the ethereal solution twice with 20 cc. of water, with vigorous shaking on each occasion.

After one or other of these preliminary treatments, wash the ethereal solution three times with 20 cc. of 0.5 *N* aqueous potassium hydroxide solution, shaking vigorously on each occasion, each alkali wash being followed by a wash with 20 cc. of water. After the last 0.5 *N* aqueous potassium hydroxide treatment, wash with two or more successive quantities of 20 cc. of water until the wash water no longer reacts alkaline to phenolphthalein solution.

Transfer the ethereal extract to a weighed flask, distill off the ethyl ether, and dry the residue to constant weight, not allowing the temperature to exceed 80° C.

In certain unusual cases the unsaponifiable matter appears to suffer a continuous loss during drying, owing to the presence of some material of low volatility, as, for example, residual solvent fractions. In such instances, transfer the washed ethereal extract to a flask containing about 2 g. of a neutral oil, such as arachis, previously brought to constant weight at 80° C. and then proceed as in the ordinary determination. Under these conditions the neutral oil serves to minimize any loss.

The drying may be aided by the use of acetone with very low non-volatile residue. When practically all the ethyl ether is evaporated, add 2 to 3 cc. of acetone. By the aid of a gentle current of air remove the solvent completely from the flask, which is preferably almost entirely immersed, held obliquely and rotated in a boiling water bath.

After attaining constant weight, dissolve the contents of the flask in 10 cc. of freshly boiled and neutralized 95 per cent alcohol and titrate with 0.1 *N* alcoholic sodium hydroxide solution, phenolphthalein solution being used as indicator. Provided that, when the determination is carried out in the above described manner, the amount of 0.1 *N* alcoholic sodium hydroxide solution required does not exceed 0.1 cc., take the unsaponifiable matter as being the amount weighed. If the quantity of 0.1 cc. of 0.1 *N*

alcoholic sodium hydroxide solution is exceeded, repeat the determination from the start, as this limit may correspond with 0.11 per cent of free fatty acid or much larger quantities of acid soap.

If there is any reason to suspect the incomplete separation of saponifiable matter, subject the material, as weighed, to resaponification, re-extraction and washing, under the conditions specified in the method. If, on this retreatment, the amount of unsaponifiable matter obtained is not the same as that weighed in the first determination, within the limits of manipulative error, ignore the whole test and repeat the determination from the beginning.

Shortened Method: It is known that with many oils and fats the method described in detail above may be shortened by reducing the number of washes with aqueous potassium hydroxide solution from three to two, and by omitting the intermediate water-washing treatments. Following the three preliminary water washes, in the shortened method wash the ethereal solution twice with 20 cc. of 0.5 *N* aqueous potassium hydroxide by shaking vigorously on each occasion, and then with two or more successive quantities of 20 cc. of water, until the wash water no longer reacts alkaline to phenolphthalein solution. The directions are then identical with those described in the full method.

Rancidity

Deterioration of fats and oils is produced by the auto-oxidation of the unsaturated components. The reaction probably consists in the addition of molecular oxygen to the double bonds of the unsaturated acids with the production of labile peroxides which then further isomerize, or decompose spontaneously into, or react with water to form a complex series of products including aldehydes, ketones and acids of lower molecular weight.

One of these products of oxidation is epihydrinaldehyde, which gives a color with phloroglucinol. It is upon this reaction that the Kreis test depends. Place 5 cc. of the fatty body into a test tube and add 5 cc. of hydrochloric acid, free from nitrosyl chloride. Stopper the tube with a clean rubber stopper and shake vigorously for 30 seconds. Add 5 cc. of 0.1 per cent ether solution of phloroglucinol, restopper and shake for 30 seconds and then allow to stand for 10 minutes or centrifuge for 2–5 minutes. If a pink or red color is present in the acid layer, proceed as follows: Make 2 mixtures of the original oil or fat, first, one part of sample and 9 parts of liquid petrolatum and secondly, one part of sample

and 19 parts of liquid petrolatum. Test 5 cc. portions of the mixtures as detailed above and note the colors produced. The fatty bodies may then be grouped into 4 classes.

1) No reaction indicates no rancidity.
2) Positive reaction when undiluted indicates no rancidity as far as taste and odor are concerned but that the fat or oil will soon turn rancid.
3) Positive reaction diluted 1 to 10 but none diluted 1 to 20 indicates incipient rancidity, often noticeable by taste and odor.
4) Positive reaction diluted 1 to 20 indicates definite rancidity.

A pink or red color indicates a positive reaction, yellows, oranges and faint pinks should be disregarded. Crude vegetable oils, for example, partially refined cottonseed oil, give a strong Kreis test, hence care must be taken in the interpretation of this test.

The Kreis reaction responds to the formation of epihydrinaldehyde by auto-oxidation of the fatty material. However, fats undergo degradation in other ways as was pointed out and the Kreis reaction may fail. The method described above may be disturbed by the presence of small amounts of allyl alcohol, allylamine, eugenol, etc., which also give a red color with phloroglucinol. Moreover, if the aldehyde content has greatly increased owing to the advanced deterioration of the fatty material, sparingly soluble, colorless phloroglucides may be formed and the Kreis reaction may fail. In order to overcome the foregoing interferences and confirm rancidity, Taufel and Sadler [9] recommend a method based on volatilizing the epihydrinaldehyde.

The oil or melted fat is mixed with an equal quantity of ice-cold hydrochloric acid. A cotton-wool plug, inserted in the tube at its upper dry part, is moistened with 1 cc. of 0.1 per cent phloroglucinol solution in alcohol and 10 drops of 20 per cent hydrochloric acid. The tube is well shaken for 1 to 2 minutes without splashing the cotton and may if necessary be gently warmed to 40° C. A red coloration at the lower surface of the cotton-wool indicates epihydrinaldehyde.

Lea [10] states that aliphatic aldehydes of medium molecular weight, particularly heptaldehyde and nonaldehyde are compounds mainly responsible for the objectionable odor and flavor of oxidized fats and oils.

The rancidity of oils and fats may also be ascertained by a determination of the peroxide number as described under, "Fish," Chapter XV.

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[9] Taufel and Sadler, *Z. Untersuch. Lebensm.* **67**, 268 (1934).
[10] Lea, *Ind. Eng. Chem., Anal. Ed.* **6**, 241 (1934).

Peanut Oil

Peanut oil, arachis oil, earthnut oil is the edible oil obtained from the peanut. It contains about 5 per cent arachidic acid as the glyceride. Arachidic acid is insoluble in cold alcohol in comparison to stearic and palmitic acids. The Bellier [11] and Renard [12] tests are based on this fact. The Evers-Bellier [13] test for peanut oil in other oils uses the following technique. One cc. of the oil is saponified with 5 cc. of 1.5 *N* alcoholic potassium hydroxide solution by heating on a water bath for five minutes, avoiding loss of alcohol. Add 50 cc. of 70 per cent alcohol and then 0.8 cc. of hydrochloric acid, sp. gr. 1.16. After heating to dissolve any precipitate that may be formed the solution is cooled in water, stirring continuously with a thermometer, so that the temperature falls at the rate of about 1° C. per minute. If a turbidity appears before the temperature reaches 9° C., it is best to check the presence of arachis oil by isolating the acid and determining the melting point. If the liquid remains clear at this temperature, arachis oil may be regarded as absent.

It is essential that the stirring should be continuous, since local cooling will cause the premature formation of a turbidity. For this reason the cooling water should not rise above the level of the liquid in the flask. The turbidity is best observed by looking through the liquid against a good light, and noting the temperature at which a definite precipitate first appears. The point is quite sharp and the personal error should not be more than ±0.25° C. Occasionally after acidification an oil gives a slight opalescence which is unaffected by warming. This may be disregarded, as it does not affect the true turbidity temperature.

Cottonseed Oil

Cottonseed oil is the edible oil obtained from the seed of the cotton plant, or from the seed of other species of cotton plant. Cottonseed oil may be identified by use of the Halphen [14] reagent. This reagent consists of a 1 per cent solution of sulfur in carbon bisulfide to which an equal volume of amyl alcohol is added. To 10 cc. of sample in a test tube, add 10 cc. of reagent and some powdered pumice to prevent bumping. Heat in a salt water bath on a hot plate for at least one hour. A deep

[11] Bellier, *Ann. chim. anal.* **4**, 4 (1899).

[12] Renard, *Compt. rend.* **73**, 1330 (1871).

[13] Evers, *Analyst* **62**, 96 (1937).

[14] Halphen, *J. pharm. chim.* [6] **6**, 390 (1899).

red coloration shows the presence of cottonseed oil. Cottonseed oil subjected to heat circa 250° C. for 10 minutes fails to give this reaction. Kapok oil also gives the red coloration with the Halphen reagent.

This test is often used to detect adulteration of butter and lard with cottonseed stearin. However, butter and lard obtained from cows and hogs fed on cottonseed cakes or meal also give a positive reaction. Hence care must be taken in the interpretation of results. It is well to run controls containing 1 to 10 per cent of cottonseed oil in olive oil or other oil or fat on which the test is being performed. These controls give some measure of the production of light color standards by small amounts of cottonseed oil. They are necessary, for some pure olive oils, especially Tunisian oils give a faint coloration, which, however, is not comparable to the color obtained from the controls.

Villavecchia Test for Sesame Oil

Furfural reacts with the sterols peculiar to sesame oil to give a red coloration. This fact is used as the basis for a test showing the presence of sesame oil in other oils. Prepare a 2 per cent solution of furfural in alcohol. To 5 cc. of oil in a test tube, add 2–3 drops of furfural solution and 5 cc. of hydrochloric acid. Shake vigorously for 30 seconds and allow the mixture to separate. A deep red coloration in the acid layer indicates the presence of sesame oil. Confirm by addition of 5 cc. water and shaking again. If the color is due to sesame oil, the color will remain in the acid layer whereas that due to other oils will disappear. If the oil or fat be colored with an aniline dye the hydrochloric acid will extract this coloring matter and yield a pink coloration. However, this coloring matter will generally go back into the fat layer on dilution with water. As little as 2 per cent of sesame oil will give a much deeper red color than is usually obtained from the small amount of dye.

The same precautions must be observed in interpretation of results obtained with this test as that with the Halphen test, for cows and hogs fed on sesame cakes yield butter and lard giving a positive Villavecchia or Baudouin reaction.

Nitric Acid Test

A test often applied to olive oil to detect adulterants is the nitric acid test. This reaction must be interpreted cautiously and at best is only

indicative of adulteration. However, gross adulterations will respond to this simple sorting test. To 5 cc. of oil in a test tube add an equal volume of nitric acid (9 : 1). Shake thoroughly for 2 minutes and allow to stand. Pure olive oil remains unchanged, whereas a brown coloration indicates the presence of a foreign oil. Some olive oils give a light brown color. This reaction was formerly used often as a means of indicating the presence of cottonseed oil in olive oil.

Fitelson [15] Test for Tea Seed Oil

The chemical composition of olive oil and tea seed oil as far as the glycerides are concerned is very similar. They differ somewhat in the character of the sterols present in the unsaponifiable matter of the oils. Chloroform, acetic anhydride, and sulfuric acid added to tea seed oil produce a deep fluorescent color, green by reflected light and brown by transmitted light which changes to an intense red color on the addition of *anhydrous* ethyl ether and this color finally fades to a light brown. Olive oil and other common edible oils show a green color but none of these oils show the red color on the addition of the ether, although some olive oils show a faint pink before fading to the final light brown.

Measure into a test tube exactly 0.8 cc. of acetic anhydride, 1.5 cc. of chloroform and 0.2 cc. of concentrated sulfuric acid. Mix and cool to room temperature. Add 7 drops of the oil to be tested directly to the reagents, mix and cool again. To measure the 7 drops of oil use glass tubing, 4 mm. outside diameter and approximately 2 mm. inside diameter. These 7 drops should weigh approximately 0.22 g. If the solution of oil in the reagents is cloudy after mixing and cooling, add acetic anhydride dropwise, shaking after each addition until a clear solution is suddenly formed. Appreciable deviations from these quantities, particularly in the sulfuric acid, cause distinct variations in color intensities. Since the mixed reagent deteriorates slowly, do not mix in advance of testing.

After the test tube and contents have remained at room temperature for 5 minutes, note the color produced. Tea seed oil will exhibit a deep green by reflected light and brown by transmitted light. Olive oil will show a green color by reflected and transmitted light, occasionally exhibiting a faint fluorescence. Add 10 cc. of anhydrous ethyl ether from a graduated cylinder and mix immediately by inverting once. Tea seed

[15] Fitelson, *J. Assoc. Official Agr. Chem.* 19, 493 (1936).

oil will show a brown color changing to an intense red within a minute or so. This red color reaches a maximum and then fades slowly within a period of a few minutes. Olive oil forms an initial green color on addition of the ether. This color fades slowly to a brown-gray, occasionally passing through a faint pink stage. Mixtures of olive oil and tea seed oil show the characteristic tea seed oil colors proportional in intensity to the quantity of tea seed oil present.

For approximately quantitative estimations, drop the oil into the reagents as described above and allow to remain at room temperature for 5 minutes. In the meantime, cool a 10 cc. portion of anhydrous ether in ice water. At the end of the 5 minute period place the test tube containing the oil and reagents in the ice water for 1 minute, add the cold ether, taking care that no water falls into the test tube, and mix. Return the tube to the ice water bath and allow the colors to reach a maximum within 5 minutes. This maximum intensity will remain stable for 5–10 minutes before beginning to fade. Use the deepest colors for comparison. Standards containing known quantities of tea seed oil in an olive oil that gives no pink color with this test, should be run simultaneously with the sample.

Valenta Test

The Valenta test modified by Fryer and Weston [16] determines the temperature at which glycerides are soluble in acetic acid or in a mixture of amyl alcohol and ethyl alcohol. The temperature at which a turbidity first appears on cooling the mixture is ascertained. Rape oil has the highest Valenta value and coconut oil has the lowest Valenta value of the common edible oils and fats.

Triacetin

Fincke [17] gives a method for the detection of the addition of synthetic triacetin to butter or butter substitutes by use of the fact that it is soluble in dilute alcohol. Determine the Reichert-Meissl value of a portion of the sample in the usual manner. Transfer about 30 g. of the fat to a suitable flask, add 150 cc. of water, 150 cc. of 95 per cent alcohol, and some

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[16] Fryer and Weston, *Analyst* 43, 4 (1918).
[17] Fincke, *Z. Nahr. Genussm.* 11, 666 (1908).

pumice stone. Reflux for an hour. Transfer the mixture to a separatory funnel. Allow the layer to separate and cool. Draw off the water layer. Transfer the fat to an evaporating dish and evaporate off the alcohol. Dry the fat. Determine the Reichert-Meissl number of the treated fat. If this value of the Reichert-Meissl is lower than the value previously obtained, triacetin or other material soluble in alcohol and contributing to the Reichert-Meissl value is present in the original sample. If the values obtained are substantially the same, no such material is present in the original sample.

Interpretation of Results

In the main, as was stressed previously, these determinations and color tests are used as a means for ascertaining whether butter and olive oil are pure. Deviation of one constant alone from the normal is not sufficient to substantiate belief of adulteration. Thus, if the Reichert-Meissl of a butter was 17 and the other factors were normal, this might well be an abnormal variation of a pure butter.

Divers adulterants produce different variations in the characteristic constants of butter and olive oil. Table 12 shows the increase or decrease in the constants of butter fat by the substitution in whole or in part by coconut oil, palm kernel oil, beef tallow, lard and hydrogenated cottonseed.

Oleomargarine is essentially a mixture of varying amounts of coconut oil and/or palm kernel oil with beef tallow and/or lard and/or hydrogenated cottonseed plus cottonseed stearin, plus a small amount of sweet cream or butter to flavor and aid in homogenization of the margarine. The constants of margarine vary, then, according to the mode of manufacture.

In general, the smallest adulteration that can be estimated with a fair degree of accuracy is approximately 10 per cent. Therefore small increases or decreases as outlined in Table 12 must be interpretated with caution. Exact estimation of the percentage is not possible. However, close approximation may be given by calculation on the basis of normal average values of the constants of the oils involved.

Table 13 shows the increase or decrease in the constants of olive oil by substitution in whole or in part by the following common adulterants, cottonseed, corn, peanut, sesame, soya, sunflower, rape and tea seed. The color tests and other tests by which these foreign oils may be detected are named.

TABLE 12. INCREASE OR DECREASE IN CONSTANTS OF ADULTERATED BUTTER FAT

Name	Refractive Index	Reichert-Meissl Value	Polenske Value
Coconut	—	—	+
Palm kernel	—	—	+
Beef tallow	+	—	—
Lard	+	—	—
Hydrogenated cottonseed	+	—	—

Name	Iodine Value	Saponification Value	Hehner Value
Coconut	—	+	little change
Palm kernel	—	+	little change
Beef tallow	+	—	+
Lard	+	—	+
Hudrogenated cottonseed	+[18]	—	+

[18] Can be controlled to any iodine value depending on the degree of hydrogenation.

TABLE 13. INCREASE OR DECREASE IN CONSTANTS OF ADULTERATED OLIVE OIL

Name	Refractive Index	Specific Gravity	Iodine Value
Cottonseed	+	+	+
Peanut	+	no change	+
Sesame	+	+	+
Rape	+	slight change	+
Tea seed	no change	no change	no change
Soya	+	+	+
Sunflower	+	+	+
Corn	+	+	+

Name	Saponification Value	Specific Tests
Cottonseed	no change	Halphen
Peanut	no change	Bellier, Renard
Sesame	no change	Villavecchia
Rape	decrease	Valenta
Tea seed	no change	Fitelson
Soya	no change	
Sunflower	no change	
Corn	no change	odor

REFERENCES

Jamieson, "Vegetable Oils and Fats," Chemical Catalog (1932).
Cox, "Analysis of Foods," Blakiston (1926).
Fryer and Weston, "Oils, Fats and Waxes," Cambridge (1920).
Allen's Commercial Organic Analysis, 5th ed., Vol II, Blakiston (1924).
Lewkowitsch-Warburton, "Oils, Fats and Waxes," Macmillan (1922).
Methods of A. O. A. C. (1935).
Elsdon, "Edible Fats and Oils," Van Nostrand (1926).
Report of Committee on Analysis of Commercial Fats and Oils, Am. Chem. Soc., Ind. Eng. Chem., Anal. Ed., **8**, 233 (1936).
Fieser, "Chemistry of Natural Products Related to Phenanthrene," Reinhold (1936).

CHAPTER IX

SUGAR FOODS AND CARBOHYDRATES

THE food analyst seldom needs to analyze carbohydrates, that is substances similar to sugar, syrups and starches, as such, except for maple sugar, maple syrup and commercial glucose. Generally, his object is to determine the amount and kind of carbohydrate in various food products. Thus for example, the analyst is interested in the sugar content of such different food materials as fruits, fruit juices, vegetables, vegetable juices, jams, preserves, jellies, honey, confectionery, milk, preserved meats and many other products.

The carbohydrates encountered in foods may be classified into a number of groups:

I. Monosaccharides

1. *Pentoses*
    a) arabinose
    b) xylose
2. *Hexoses*
    a) dextrose — D-Glucose
    b) levulose — D-Fructose
    c) galactose

II. Disaccharides

a) sucrose
b) lactose
c) maltose

III. Polysaccharides

1. *Trisaccharide*—raffinose
2. *Dextrins*
    a) amylodextrins
    b) maltodextrins

3. *Starches*
   a) starch
   b) inulin
   c) glycogen
4. *Miscellaneous*
   a) gums
   b) pectins
   c) hemicellulose
   d) cellulose

The problem of estimating the sugar content of a food product resolves itself no matter what the food product may be, into obtaining a water solution of the sugar or mixture of sugars free of interfering substances, upon which solution the identification tests and quantitative test may be performed. This may be done by the use of a group of substances termed clarifiers.

Clarifiers

The function of these clarifiers is to throw out of solution, which process is called defecation, substances that might interfere in determining the optical rotation, reducing power or other physical or chemical properties of sugars.

Alumina Cream—Prepare a cold saturated solution of ammonia alum in water. Add ammonium hydroxide with constant stirring until the solution is alkaline to litmus, allow the precipitate to settle, and wash by decantation with water until the wash water gives only a slight test for sulfates with barium chloride solution. Pour off the excess of water and store the residual cream in a stoppered bottle. Alumina cream is not a good clarifying agent for very dark solutions but is very useful as an additional clarifier and as an aid in filtering. Generally 1 to 2 cc. are used in addition to the other agent.

Basic Lead Acetate Solution—Boil 430 g. of neutral lead acetate, 130 g. of litharge, and 1 liter of water for 30 minutes. Allow the mixture to cool and settle and then dilute the supernatant liquid to a specific gravity of 1.25 with recently boiled water. Solid basic lead acetate may be substituted for the normal salt and litharge in the preparation of the solution. This clarifier is used mainly for dark colored solutions of

sucrose to be estimated polarimetrically. From 1.5 to 10 cc. of the agent is used according to the depth of color of the material being clarified. It is not to be used for lactose determinations nor for sucrose estimated by chemical methods.

Dry Basic Lead Acetate, Horne's Method—A pinch of the dry salt is added to the sugar solution after completion to volume and then the mixture is shaken. Add more salt and shake again, repeating the addition until the precipitation is complete, but avoid any excess. This clarifier is used for solutions similar to those in which basic lead acetate solution is used and about 1/3 g. of the dry salt is taken as equivalent to 1 cc. of the basic lead acetate solution.

Neutral Lead Acetate Solution—Add to a solution of the sample being analyzed sufficient saturated neutral lead acetate solution to precipitate completely interfering substances. Shake and make to volume. This clarifier must be used for polariscope determinations whenever reducing sugars are to be estimated in the solution for polarizing and is sometimes used for lactose determinations. Generally 3 to 4 cc. of the reagent is sufficient.

Basic Lead Nitrate, Herle's Method—This clarifier consists of two solutions. 1) Dissolve 250 g. of lead nitrate in water and make to a volume of 500 cc. 2) Dissolve 25 g. of sodium hydroxide in water and make up to 500 cc. Add equal volumes of both solutions to the solution to be clarified, shake and add more if clarification is not complete. This clarifier is used in place of basic lead acetate solution and about 3 cc. of each reagent is generally taken for this purpose.

Phosphotungstic Acid—Sometimes the dry acid is used as described above for dry basic lead acetate but more often a 20 per cent aqueous solution is used. If much reducing substances are present as in the case of meats where creatinine and like substances are present, a 1:1 aqueous solution may be found adequate.

Copper Sulfate—Lactose solutions obtained from milk and milk products are generally clarified by the use of a solution of copper sulfate, 34.639 g. of $CuSO_4.5H_2O$ dissolved in water, diluted to 500 cc. and filtered through an asbestos mat; and 0.5 N sodium hydroxide solution. They are added in the proportion of 10 cc. of copper sulfate solution to 8.8 cc. of the alkali solution.

The above mentioned clarifying agents are the ones generally used in food analysis. The use of these clarifiers produces three types of errors. Those due to the volume of the precipitate produced, those due to precipitation of sugars from solution, and those due to change in specific rotation. To keep these errors to a minimum, a minimum amount of clarifying agent should be used.

The amount of clarifier to be used is governed by the depth of color and quantity of organic material other than sugars that are present and that must be precipitated in order not to interfere with the estimation of the sugar. The general procedure in the use of these agents is to add a small quantity, say 1 to 2 cc. of the solution of the clarifier, or a pinch or spatula-tipful of a dry defecating agent to the solution being analyzed. Mix and allow the precipitate formed to settle. Add a few drops or a few grains of the agent to see if precipitation is complete. If not, the process is continued to complete precipitation. The procedure is in reality one of trial and error. Experience soon reveals the proper agent to use and the proper quantity of agent to be added.

It may be noted that 10 per cent sodium tungstate solution, 10 g. $Na_2WO_4.2H_2O$ dissolved in water and made up to 100 cc. and 2/3 N sulfuric acid are used to clarify blood on which determinations of sugar are made. Equal volumes of the reagents are used.

Calcined charcoal and allied adsorbing carbons should not be used as clarifying agents because they may adsorb some sugar and produce serious errors in the determinations.

In biochemical determinations the use of clarifiers has reached a far more advanced state than in food chemistry. The author cannot completely recommend these clarifiers because insufficient work has been done in using them in food analyses. They should, however, be fully investigated for they are based on the principle of mutual precipitation of the added salts, leaving the solution to be tested not only free of the materials for whose elimination the clarifier was added but also leaves the solution free of the clarifier itself.

Steiner, Urban and West [1] recommend ferric sulfate or thorium sulfate and barium carbonate:

$$Fe_2(SO_4)_3 + 3BaCO_3 \rightarrow Fe_2(CO_3)_3 \downarrow + 3BaSO_4 \downarrow$$

$$Th(SO_4)_2 + 2BaCO_3 \rightarrow Th(CO_3)_2 \downarrow + 2BaSO_4 \downarrow$$

[1] Steiner, Urban and West, *J. Biol. Chem.* **98**, 289 (1932).

West, Scharles and Peterson [2] recommend mercuric sulfate and barium carbonate:

$$HgSO_4 + BaCO_3 \rightarrow HgCO_3 \downarrow + BaSO_4 \downarrow$$

Kleiner and Tauber [3] suggest the use of copper sulfate and barium hydroxide:

$$CuSO_4 + Ba(OH)_2 \rightarrow Cu(OH)_2 \downarrow + BaSO_4 \downarrow$$

Of course, these substances must be added in equivalent solutions. Another clarifier, is that used by Moir and Hinks,[4] and consists of zinc acetate and potassium ferrocyanide. Its use is described in the section "Total Alkaloids," Chapter XIII.

Monosaccharides

Dextrose—Dextrose is a hexose sugar whose classification name is d-glucose. It is widely distributed being present uncombined in the blood of animals and in the juices of plants. The structure and chemistry of dextrose is very complex for it is very seldom in the free aldehyde form. It may form butylene, amylene, propylene and ethylene oxide rings which in turn can exist in an α and β configuration. In freshly prepared solutions of α dextrose the α form of a predominent ring exists alone and the sugar exhibits a specific rotation of +113.4°. As the solution stands, the rotatory power changes until it becomes constant at +52.3°. This phenomenon of slow change of rotatory power of sugars until an equilibrium is reached is termed mutarotation. It is due to the fact that when the sugar is dissolved, the form which predominates α glucopyranose slowly changes to the other form in part. At equilibrium, although the change is still going on, as many molecules of α form change into the β form as the β form change into the α form, therefore the rotation remains the same.

Dextrose is one of the end products of the hydrolysis of many polysaccharides. Starch, dextrins, raffinose, sucrose, maltose, and lactose are composed wholly or partially of dextrose residues. It may also be obtained from a group of substances called glucosides, for example salicin and amygdalin. It is a reducing sugar and hence may be identified by tests for reducing sugars.

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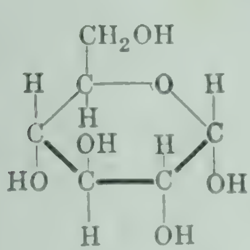
[2] West, Scharles and Peterson, *J. Biol. Chem.* 82, 137 (1929).

[3] Kleiner and Tauber, *J. Biol. Chem.* 100, 749 (1933).

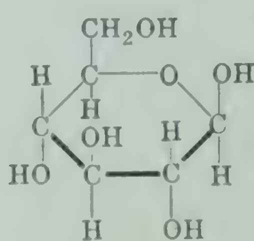
[4] Moir and Hinks, *Analyst* 60, 439 (1935).

Dextrose must not be confused with the product termed commercial glucose. Commercial glucose, or mixing glucose, is a thick syrupy, colorless product made by incompletely hydrolyzing starch or a starch containing product which is decolorized and evaporated.

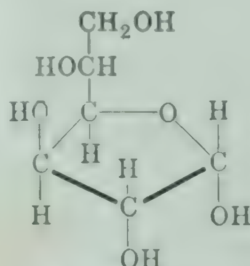
Formerly it was considered that the more stable form of α or β dextrose contained the butylene oxide ring structure and the less stable γ dextrose contained the propylene oxide structure. The work of Haworth[5] and his collaborators, however, shows that the more stable dextrose contains an amylene oxide structure and the less stable glucose contains the butylene oxide structure formerly ascribed to stable dextrose. Haworth terms these forms of dextrose as α and β glucopyranose and α and β glucofuranose, thus indicating the relationship between these sugars and the pyrane and the furane rings. The corresponding isomers of levulose are called fructopyranose and fructofuranose. Sucrose, according to Haworth, is probably a compound of glucopyranose and fructofuranose.



α-glucopyranose



β-glucopyranose

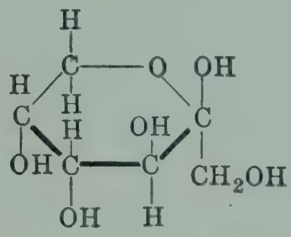


glucofuranose

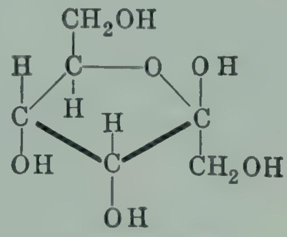
Levulose—Levulose is a ketose hexose sugar whose classification name is d-fructose. This sugar is laevorotatory and has a specific rotation of $-93°$. It is found in many plant juices, probably as a hydrolysis product of sucrose. It is formed along with dextrose by the inversion of cane sugar. It is the main hydrolytic product of inulin and comprises

[5] Haworth, "The Constitution of Sugars," Arnold (1929).

about 40 per cent of honey. It is the only important ketose sugar met with in foods. In the Haworth terminology its forms are fructofuranose and fructopyranose.

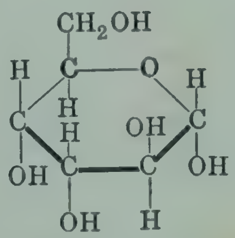


α fructopyranose



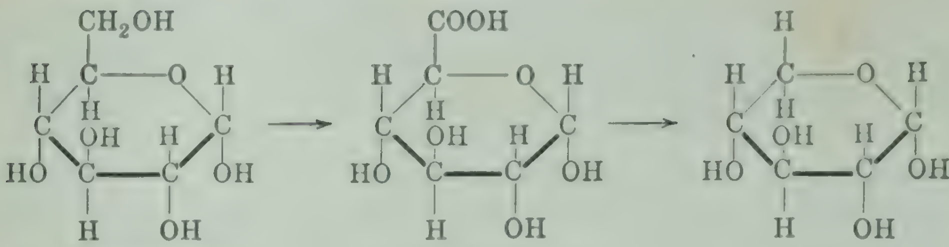
fructofuranose

Galactose—Galactose is an aldose hexose sugar that is obtained by the hydrolysis of milk sugar or lactose, galactans and gums. Its classification name is d-galactose and has a specific rotation of +81.5°. It forms on oxidation under proper conditions, a characteristic acid, namely, mucic acid, which is not formed by other sugars or carbohydrates that do not contain galactose residues. It forms, as do the aforementioned sugars, galactopyranose and galactofuranose.



galactopyranose

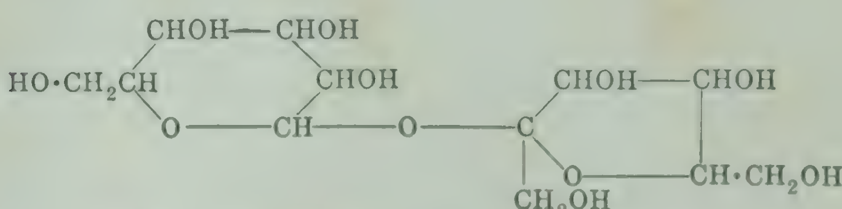
Pentoses—Arabinose, xylose, ribose, lyxose and rhamnose are examples of pentose sugars and are the hydrolytic products of pentosans, nucleo-proteins, gums and similar materials. Of these arabinose and xylose are most important to the food analyst. They have strong reducing power and are non-fermentable. Ribose, as will be detailed in the chapter on vitamins, forms a portion of vitamin B_2, lactoflavin. These sugars yield furfural on prolonged boiling with hydrochloric acid. Their pyrane ring structures show their similarity to dextrose and the hexose sugars in general.



α-glucopyranose *d*-glucose — *d*-glycuronic acid — xylose

Disaccharides

Sucrose—Sucrose is a sugar characteristic of plant materials. It may be obtained from sugar cane, sugar beet, sugar maple and other plants. On hydrolysis it yields dextrose and levulose in equimolecular quantities. The syrup formed by the hydrolysis of raw cane sugars is known as invert sugar. Sucrose is dextrorotatory and is a non-reducing sugar. The invert sugar formed by hydrolysis is laevorotatory because levulose is more strongly laevorotatory than dextrose is dextrorotatory. Haworth states that sucrose is a compound of α glucopyranose and fructofuranose. In the hydrolysis the fructofuranose changes to fructopyranose.

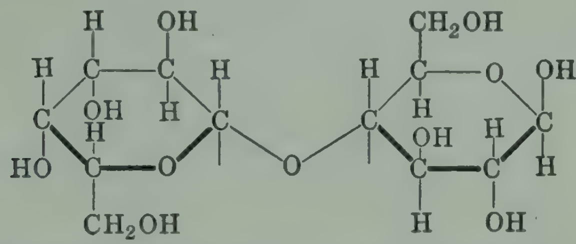


glucopyranose — fructofuranose

Sucrose

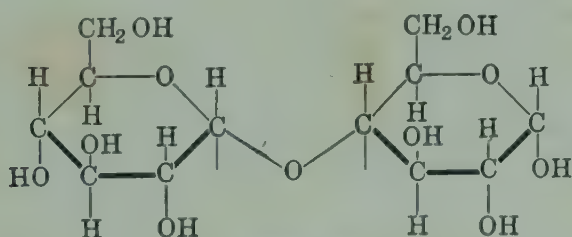
Lactose—Lactose is a sugar that is produced by animals and is obtained from the milk of mammals. On hydrolysis, it yields equimolecular quantities of dextrose and galactose. It is not hydrolyzed as easily as sucrose. It is a reducing sugar but is not fermentable by baker's yeast and thus may be separated by fermentation from dextrose and similar fermentable sugars. Since one of its hydrolysis products is galactose, lactose also yields mucic acid on oxidation with nitric acid. Lactose is

dextrorotatory and has a specific rotation of +52.5°. According to Haworth it is β glucopyranose-β galactopyranose.



β-galactopyranose β-glucopyranose
Lactose

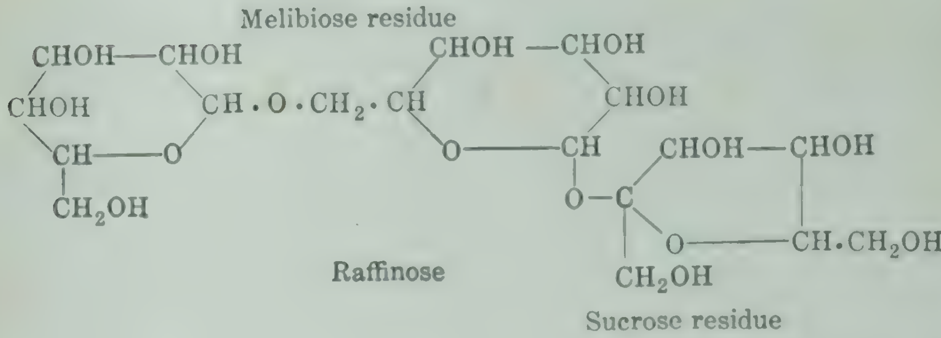
Maltose—Maltose generally does not occur free in nature. It is formed by the action of enzymes of plants and animals on starch. It may also be formed by the action of acids on starch. The further hydrolysis of maltose yields dextrose. Maltose is present in commercial glucose, beer, malted milk powder and like products. Maltose is strongly dextrorotatory having a specific rotation of +137°. It is also a reducing sugar. Maltose is fermentable by yeast. It is an α glucoside, more specifically, α-glucopyranose-α-glucopyranose.



Maltose

Polysaccharides

Raffinose—Raffinose is a trisaccharide and yields on hydrolysis a molecule each of dextrose, levulose, and galactose. It is dextrorotatory. It is a non-reducing sugar. On special hydrolysis it yields, at times, a melibiose residue and at other times a sucrose residue. Hence, it gives the Raybin diazouracil reaction (refer to page 251) and because melibiose contains galactose, the mucic acid reaction. It is obtained in the manufacture of sugar from sugar beet.



Dextrin—Dextrin is a product of the conversion of starch. This conversion may be made by acids or enzymes. Amylodextrin is the first stage of this conversion and is known as soluble starch. This stage still gives a blue color with iodine solution. A further stage of hydrolysis is termed erythrodextrin and yields a red color with iodine solution. Maltodextrin is a stage of conversion at which no further color is obtained with iodine solution.

Starch—Starch is a polysaccharide whose exact molecular formula is unknown. It is composed of carbon, hydrogen and oxygen in the proportion $C_6H_{10}O_5$. It is found in the roots, tubers, grains and seeds of plants. The small white starch granules have a characteristic form for each type of plant and these structures may be identified microscopically. It is insoluble in cold water, alcohol and ether but swells up and forms a paste with hot water. It is hydrolyzed by enzymes and acids to dextrins, maltose and dextrose. Final hydrolysis yields dextrose. Starch gives the well known characteristic blue color with iodine.

Inulin—Inulin is another polysaccharide of plant extraction with a high molecular weight $(C_6H_{10}O_5)_x$. It is obtained from the tubers of the artichoke and dahlia and from the roots of chicory and dandelion. It is slightly soluble in cold water and easily soluble in hot water. It does not give a red or blue color with iodine and on hydrolysis yields levulose in contradistinction to starch. Inulin is laevorotatory.

Glycogen—Glycogen is a carbohydrate of animal extraction and of high molecular weight $(C_6H_{10}O_5)_x$. It is found in the liver and in small amounts in other parts of the body. It is a white amorphous powder, which is soluble in water yielding a dextrorotatory solution. It is non-

reducing and gives a red color with iodine. It may be hydrolyzed in a manner similar to starch by enzymes such as diastase forming dextrins, maltose and dextrose. The muscle of horse meat contains more glycogen than is found in the muscle of other animals and hence the presence of glycogen in meat products is an indication of horse meat.

Chemical Reactions

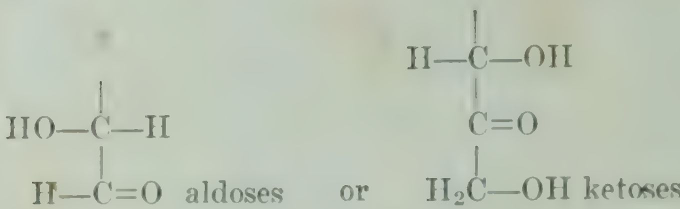
Before proceeding with the quantitative determination of sugars and carbohydrates in general, it is best to ascertain the kind of carbohydrate present qualitatively. Of course, the food product itself often gives the clue unless it be grossly adulterated. Thus milk and milk products except those sweetened with sucrose, as for example, sweetened condensed milk and ice cream and maltose in the case of malted milk, will contain lactose and a determination of the sugar reducing power may be interpreted as being due to a definite quantity of lactose. In a like manner, the reducing sugars of malt beverages such as beer, ale, etc., are calculated as maltose.

Sugars and carbohydrates give color reactions with many phenols. Alpha naphthol, thymol, resorcinol and phloroglucinol are some of the phenols used. Alpha naphthol is the one most often used and this reason is known as the Molisch reaction. In this reaction the furfural that is formed by the sulfuric acid condenses with the phenol to give the characteristic color.

Molisch Reaction—Place about 5 cc. of concentrated sulfuric acid into a test tube. Add 2 drops of a 15 per cent alcoholic solution of α naphthol to 5 cc. of the solution to be tested. Carefully overlay the sulfuric acid with the 5 cc. of solution to be tested containing the Molisch reagent. A reddish-violet ring produced at the interface of the two liquids shows the presence of a carbohydrate. This test is not specific for it is given by all the members of the carbohydrate group capable of yielding furfural in traces.

Reducing Power—The sugars are classified chemically into two groups, the reducing sugars and the non-reducing sugars. Dextrose, levulose and galactose, lactose and maltose are reducing sugars. Sucrose and raffinose are non-reducing sugars. The chemical properties of these

reducing sugars are due to the presence of carbonyl-alcohol group in the molecule:



aldoses or ketoses

If the terminal groups are aldehydes-alcohols, the sugars are known as aldoses and if the terminal groups are ketones-alcohols, the sugars are known as ketoses. Dextrose is an aldose. Levulose is a ketose.

This reducing power is evidenced by the action of solutions of sugars on alkaline solutions of copper, silver, mercury and bismuth. The alkaline solutions of copper have had the most widespread use, and of these the reagents of Fehling, Benedict, Barfoed, Tauber and Fischl are taken as examples.

Fehling Test. This test is based on the reduction of an alkaline copper tartrate solution with the formation of red to yellow cuprous oxide by sugar solutions. The Fehling reagent is composed of two solutions which are mixed immediately prior to the making of the test. 1) Copper sulfate solution: 34.65 g. of $CuSO_4.5H_2O$ is dissolved in water made up to 500 cc. and filtered. 2) Alkaline tartrate solution: 125 g. of potassium hydroxide and 173 g. of sodium potassium tartrate (Rochelle salts) dissolved in water made up to 500 cc. and filtered through asbestos. Both solutions are kept in separate bottles.

Add to 1 cc. of the mixed Fehling reagent in a test tube, 4 cc. of water. Boil or better heat in a boiling water bath and see if a precipitate forms. If one does form, the solutions must be discarded and fresh solutions must be made. To the warm 5 cc., if no precipitate forms, add a few drops of the sugar solution which has previously been clarified with neutral lead acetate and deleaded with potassium oxalate as will be explained in the quantitative methods. Boil or preferably heat in a boiling water bath and if necessary add a few more drops of the sugar solution. Heat after each addition and do not add a total of more than 10–12 drops. A yellow to brick red precipitate is produced in the presence of reducing substances. This test is not specific and will be given by any of the reducing sugars.

Benedict Test. This test is based on the reduction of an alkaline copper citrate solution with the formation of red, yellow or green precipitates

of cuprous oxide. This reagent has the marked advantage that only one solution is needed and that creatinine and uric acid do not interfere markedly. Dissolve 173 g. of sodium citrate and 100 g. of anhydrous sodium carbonate in 800 cc. of water with the aid of heat. Filter. Dissolve 17.3 g. of copper sulfate, $CuSO_4.5H_2O$ in about 100 cc. of water and filter. Make up the citrate-carbonate solution to 850 cc. and place the solution in a beaker. Add the copper sulfate solution slowly and with constant stirring. Make the mixture up to 1 liter. This reagent does not deteriorate on long standing.

To 5 cc. of the Benedict reagent in a test tube add 8 drops of the solution to be tested and boil. Continue boiling for 2 minutes and note the formation of a colloidal colored precipitate. If no precipitate forms, reducing sugars are absent. The sugar solution should be prepared by clarifying with neutral lead acetate and the excess lead should be removed by potassium oxalate. Small quantities of sugar such as dextrose will give a precipitate with this reagent. This test is not specific and will be given by any of the reducing sugars.

Barfoed Test. This test is based on the reduction of a slightly acid solution of copper acetate. Dissolve 13.3 g. of neutral crystallized copper acetate in 200 cc. of water. Add 1 cc. of glacial acetic acid and allow the mixture to stand. Use the clear supernatant liquid.

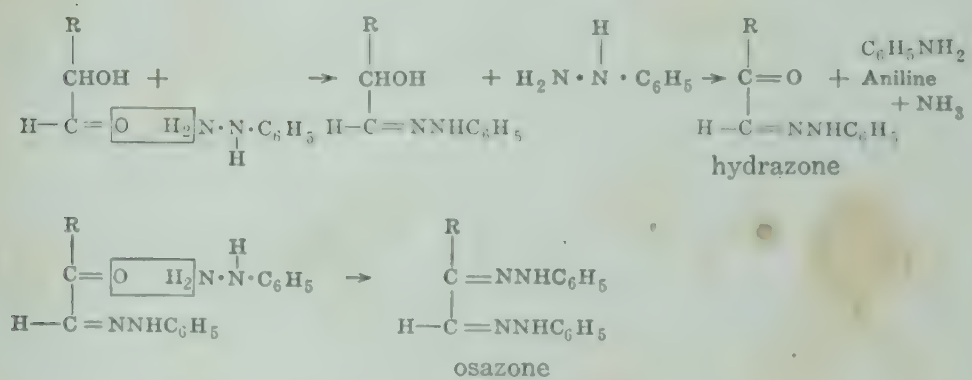
To 5 cc. of the reagent, add 1 cc. of the solution to be tested. Place in a boiling water bath for 3 minutes. At the end of this period note the formation of any red precipitate on the sides or bottom of the tube. If no precipitate has formed, place aside for a few minutes and examine again. A red precipitate shows the presence of a monosaccharide. Lactose and maltose will produce a precipitate only after prolonged boiling. Hence this reagent may be used to distinguish between monosaccharide and disaccharide reducing sugars. If reduction is obtained with either the Fehling or Benedict reagent and none with the Barfoed reagent, a disaccharide is indicated. This is the basis of a qualitative test for the detection of milk products in foods as described in Chapter VII.

If chlorides are present in the solution to be tested, a green precipitate or a whitish precipitate may form. This is not to be mistaken for a positive sugar reduction. Phosphotungstic acid cannot be used as a clarifying agent if this test is to be performed, for potassium chloride is used to precipitate the excess phosphotungstic acid.

Osazone Reaction—The reducing sugars have the property of reacting with phenylhydrazine under definite conditions to form difficultly

soluble substances called osazones. These compounds can be easily purified and melting points can be obtained. Hence, they are useful in the identification of sugars. To a mixture of 1 g. of phenylhydrazine hydrochloride and 1.5 g. of sodium acetate add 5 cc. of the sugar solution and place the tube containing the mixture in a boiling water bath for 45 minutes. Allow the tube to cool slowly. The osazones of the monosaccharides crystallize from hot solution whereas those of the disaccharides crystallize on cooling, consequently the crystals may be separated by filtration.

Although the osazones can be recrystallized and a melting point obtained, the melting points lie so closely together that the osazones are best identified by their characteristic crystalline structure with the aid of a microscope. If the phenylhydrazine solution becomes too concentrated, the osazone may not crystallize out until it is diluted with water. Dextrose and levulose yield the same osazone because they have the same structure in the part of the molecule unaffected by the osazone reaction. The reaction goes in the following manner:



Seliwanoff Reaction—This reaction is based on the formation of a red color by a ketose sugar in the presence of resorcinol and hydrochloric acid. The reagent is prepared by dissolving 0.05 g. of resorcinol in 100 cc. of hydrochloric acid (1:2). To 5 cc. of the reagent in a test tube add 1 cc. of the sugar solution and heat to boiling. A striking red color produced in less than half a minute of boiling indicates the presence of a ketose sugar. This reaction is generally considered to indicate levulose, however, sucrose will give this reaction because it is hydrolyzed rapidly in the boiling acid and yields levulose which in turn gives the red color. The red color develops into a brown-red precipitate which is soluble in alcohol.

Raybin[6] Reaction—This reaction is based on the formation of a blue-green color due to the formation of a condensation product of diazouracil and sucrose. If 5 cc. of a 0.05 *N* sodium hydroxide solution (10° C.) containing 40–50 mg. of sucrose is shaken in a corked test tube with 7–10 mg. of diazouracil until the reagent dissolves, a blue green color develops within a few minutes in the cold. Raffinose is the only other sugar giving this reaction. Other sugars and polysaccharides give yellow to brown red colors.

Mucic Acid Reaction—Galactose and any sugar or polysaccharide yielding galactose on hydrolysis, as for example lactose, raffinose and gums give mucic acid on oxidation with nitric acid. To 100 cc. of the sugar solution in a beaker with a graduation at 20 cc. add 20 cc. of nitric acid and evaporate slowly on a hot plate regulated to 80° C. until the volume of the mixture is reduced to 20 cc. Allow to stand for 24 hours; note the formation of a fine white precipitate of mucic acid. If no precipitate is formed or if quantitative estimation of galactans is being made, dilute to 100 cc. with water and add an additional 20 cc. of nitric acid. Again evaporate slowly to 20 cc. on a hot plate or steam bath. A fine white precipitate, insoluble in water but readily soluble in alkali or ammonium carbonate solution and reprecipitated on the addition of nitric acid is mucic acid. The weight of mucic acid multiplied by the factor 1.33 gives the weight of galactose.

Phloroglucinol Reaction—This reaction is based on the formation of a red to amber color by heating pentoses, glycuronic acid, galactose, or galactans with hydrochloric acid and a few crystals of phloroglucinol. To 10 cc. of solution of the carbohydrate add 10 cc. of hydrochloric acid and a few crystals. Heat rapidly to boiling. The production of a red to amber color within 2 minutes indicates the presence of the above-mentioned carbohydrates.

Bial Orcinol Reaction—The reagent is made by dissolving 1.5 g. of orcinol in 500 cc. of hydrochloric acid (sp. gr. 1.15 —30%) to which 20 drops of 10 per cent ferric chloride solution is added. Five cc. of the reagent is heated to boiling, removed from the flame and a few drops but not over 1 cc. of the solution to be tested is added. If pentoses are present, a vivid green color will be developed almost immediately. This

[6] Raybin, *J. Am. Chem. Soc.* 55, 2603 (1933).

reaction will be given by carbohydrates capable of producing pentoses. It will not be given by glycuronic acid, hence it is characteristic of pentoses.

Quantitative Methods

The quantitative methods for the estimation of sugars and carbohydrates depend on the properties of reduction and optical rotation that sugars have. The reduction methods resolve themselves to weighing the amount of cuprous oxide produced by a given quantity of sugar solution, that is, a gravimetric method; or to measuring the volume of sugar solution necessary to reduce completely a given volume of copper solution. As representative of the gravimetric methods the Munson and Walker method will be detailed and as representative of the volumetric method, the Lane-Eynon method will be described. These methods are empirical and therefore the directions must be followed exactly in order to obtain correct results.

Munson and Walker Method—The Soxhlet modification of the Fehling reagent is used as the copper reagent in this method. 1) Dissolve 34.639 g. of pure copper sulfate, $CuSO_4.5H_2O$, in water, dilute to 500 cc. and filter through asbestos washed successively with hydrochloric acid (1 : 3), 10 per cent sodium hydroxide solution and alkaline tartrate solution and then washed thoroughly with water after each treatment. This type of asbestos is termed treated asbestos. 2) Dissolve 173 g. of potassium sodium tartrate (Rochelle salt) and 50 g. of sodium hydroxide in water, dilute to 500 cc., allow to stand 2 days and filter.

The sugar solution is generally prepared for estimation by this method, by clarification with neutral lead acetate and alumina cream and the removal of excess lead with a minimum amount of anhydrous potassium oxalate.

Transfer 25 cc. of each of the copper sulfate and alkaline tartrate solutions to a 400 cc. beaker and add 50 cc. of the reducing sugar solution, or if a smaller volume of sugar solution is used, add water to make the final volume 100 cc. Keep the beaker covered with a watch glass and heat the beaker on an asbestos gauze in such a manner that boiling begins in exactly 4 minutes. Continue the heating for exactly 2 minutes more. Some practice with 50 cc. of the mixed reagents and 50 cc. of water will enable the analyst to regulate the flame properly. Filter the hot solution at once through a prepared Gooch crucible having some of the prepared asbestos for a mat. Wash the precipitate thoroughly with hot water and

place the crucible in a constant temperature oven at 100°–105° C. to dry. Cool and weigh as cuprous oxide. Determine the amount of sugar by reference to Table 1, appendix.

If the precipitate be contaminated in any way, it is best to determine the amount of copper by dissolving the cuprous oxide in nitric acid and then proceed with the iodide-thiosulfate method.

Iodide-Thiosulfate Method [7]—Standard thiosulfate solution. Prepare a solution containing 39 g. of pure $Na_2S_2O_3.5H_2O$ in 1 liter. Weigh accurately 0.2–0.4 g. of pure copper and transfer to a 250 cc. flask roughly graduated by marks at 20 cc. intervals. Dissolve the copper in 5 cc. of a mixture of equal volumes of nitric acid and water, dilute to 20 or 30 cc., boil to expel the red fumes, add a slight excess of strong bromine water, and boil until the bromine is completely driven off. Cool, and add 25 per cent sodium hydroxide solution with agitation until a faint turbidity of cupric hydroxide appears. This requires about 7 cc. of the 25 per cent sodium hydroxide solution. Discharge the turbidity with a few drops of acetic acid and add 2 drops in excess. Prepare a potassium iodide solution, 42 g. of potassium iodide in 100 cc. of solution, made very slightly alkaline to avoid formation of hydriodic acid and its oxidation products.

It is essential for the thiosulfate titration that the concentration of potassium iodide in the solution be carefully regulated. If the solution contains less than 320 mg. of copper, at the completion of the titration 4.2–5 g. of potassium iodide should have been added for each 100 cc. of total solution. If greater quantities of copper are present, add the potassium iodide solution slowly from a burette with constant agitation in amounts proportionately greater.

Observe the volume of the copper solution and add 1 cc. of potassium iodide solution for each 10 cc. of the solution undergoing titration. Titrate at once with the thiosulfate solution until the brown color becomes very faint. Again observe the volume and add an additional volume of the potassium iodide solution to make the required concentration, noting from the volume of the thiosulfate the approximate copper content of the solution. Add sufficient starch indicator to produce a marked blue coloration. Continue the titration cautiously until the color changes toward the end to a faint lilac. As the end point is approached, add the thiosulfate in fractions of drops, allowing the precipitate to settle slightly

[7] Methods Assoc. Official Agr. Chem., p. 480 (1935).

after each addition 1 cc. of the thiosulfate is equivalent to about 10 mg. of copper.

Knowing the exact amount of copper weighed and the amount of thiosulfate used in the titration the exact strength of the thiosulfate solution may be calculated.

Wash the precipitated cuprous oxide in the Gooch crucible from the Munson and Walker method. Place the crucible in the mouth of a 250 cc. flask roughly graduated at 20 cc. intervals. Cover the crucible with a small watch glass and dissolve the oxide by means of 5 cc. of nitric acid (1 : 1) directed under the watch glass with a pipette. Wash the crucible with small portions of water, using a total of 25 cc., washing the watch glass with the water. Proceed from this point as directed above, beginning from "boil to expel the red fumes." Calculate the amount of copper present from the thiosulfate titration and ascertain the amount of sugar by reference to Table 1, appendix.

Lane-Eynon Volumetric Method[8,9]—The Lane-Eynon method is a short and rapid method for the estimation of reducing sugars. Dissolve 12.5 g. of the sample in water and add 25 cc. of 10 per cent neutral lead acetate solution. Add some alumina cream and make up to 250 cc. in a volumetric flask. Shake thoroughly and filter. To 100 cc. of the filtrate, add 10 cc. of a 10 per cent solution of potassium oxalate solution. Make to 500 cc., shake and filter. Place 10 cc. of the mixed Fehling reagent, prepared as directed in the Munson and Walker Method, into a 250 cc. Erlenmeyer flask. Transfer the sugar solution to a burette, and suspend the burette over the Erlenmeyer flask which has been placed on an asbestos wire gauze. Add 15 cc. of sugar solution to the flask and heat to boiling. Boil about 15 seconds and add rapidly further portions of the sugar solution until only the faintest perceptible blue color remains. Then add 2–5 drops of a 1 per cent aqueous solution of methylene blue and continue the heating and addition of sugar solution dropwise until the titration is complete which is shown by the reduction of the dye. The flask is shaken with the aid of a flask holder during the titration and heating may be conveniently done by a suitable hot plate.

The amount of sugar may be calculated by use of the formula

$$\frac{\text{factor} \times 100}{\text{titer}} = \text{mg. of sugar in 100 cc.}$$

[8] Lane and Eynon, *J. Soc. Chem. Ind.* 42, 32T (1923).
[9] Lane and Eynon, *J. Soc. Chem. Ind.* 42, 463T (1923).

The factor is ascertained by reference to Table 2, appendix, in which the factor for each titration from 15 to 50 cc. is given. The tables give the mg. of sugar corresponding to each factor. If the reduction is complete on the addition of 15 cc. or less of solution, the 100 cc. aliquot must be further diluted. If the reduction is not complete on the addition of 50 cc., then more of the original sample must be taken for the analysis. If an accuracy greater than 1 per cent is desired, then the Fehling copper reagent should be standardized by performing a similar titration against a known amount of pure anhydrous dextrose or against a known amount of pure sucrose, inverted, as described in the subsequent section, "Sucrose by polarization before and after inversion with hydrochloric acid." If any correction is necessary it should be applied to the factors in the table. The final result is then corrected for dilution.

The sugar sample may also be prepared for this determination in the usual way with neutral lead acetate and then deleading the solution with anhydrous potassium oxalate. The analysis is then begun at the point . . . "Place 10 cc. of the mixed Fehling solution . . ."

These methods merely determine the amount of reducing sugar and in no way indicate the kind of sugar present, hence the analyst must decide which of the factors in the tables to employ. Furthermore, if sucrose is present other corrections and factors must be applied. The reader is referred to texts on sugar analysis for full explanations as to these variations.

Tauber [10] Reaction—This method may be used for the detection of monoses, that is monosaccharides, in the presence of reducing bioses, or disaccharides. The reagent used is similar to Barfoed's solution, except that the volatile acetic acid is replaced by the non-volatile lactic acid. The cuprous oxide formed in the test through the oxidation of the monoses is treated with a molybdate solution and the blue color developed is proportional to the amount of monose present. The reagent will keep unaltered, is far superior to the Barfoed reagent and can be used in quantitative work.

Reagents: 1) Copper monose reagent: 24 g. of copper acetate is dissolved in 450 cc. of boiling water. Any precipitate formed should not be filtered off. To the hot solution 25 cc. of an 8.5 per cent solution of lactic acid is added. On shaking and boiling for a short time, the precipitate

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[10] Tauber, *Mikrochemie* 14, 176 (1933-4).

is mainly dissolved. When cold, the mixture is filtered and diluted to 500 cc.

2) Benedict's[11] molybdate solution: Transfer 150 g. of molybdic acid free from ammonia to a large flask and add 75 g. of anhydrous sodium carbonate. Add water in small portions, with shaking until about 500 cc. has been added. Shake thoroughly and heat the mixture to boiling or until nearly all of the molybdic acid is dissolved. Filter, wash the residue with hot water until the total volume of the filtrate is about 600 cc. Add 300 cc. of 85 per cent phosphoric acid to the filtrate. Cool and dilute to one liter.

Method:[12] Transfer 0.5 cc. of the neutral monose-biose solution to a test tube with the aid of a pipette. This solution may be made by the usual clarification with neutral lead acetate and deleading with oxalate or sulfate. Kleiner and Tauber[13] use 4 per cent copper sulfate solution and 1 per cent barium hydroxide solution. The sugar solution should be so diluted that the total reduction is not more than 1.25 mg. per cc. or less than 0.05 mg. per cc. in dextrose equivalents, supposing the solution to contain dextrose and lactose. If the solution contains dextrose and maltose, the total reduction must not exceed 0.5 mg. per cc. Sucrose may be present up to 2.5 mg. per cc. without interfering with the test. In a second test tube 0.5 cc. of water is placed for a blank test. It is best to run a control on a known sample of the material being tested. Thus if this test is being used to distinguish between added dextrose or lactose to broken out eggs, a known sample of broken out eggs free of sugars run as a control aids in the interpretation of results. A 0.5 cc. portion of the copper-monose reagent is added to all the tubes, which are then placed for 8 minutes in a boiling water bath and then cooled for 2 minutes. A 0.5 cc. portion of the molybdate solution is added and after 2 minutes 5 cc. of water is also added. A blue color indicates monoses and is proportional to the amount present. Chlorides and some other inorganic salts interfere but as much as 1.5 mg. per cc. of sodium chloride will not.

Fischl[14] Ketose Reaction—This method may be used for detecting and determining levulose in the presence of dextrose, other aldoses and sucrose. When sugar solutions are treated under definite conditions with

[11] Benedict, *J. Biol. Chem.* 92, 141 (1931).

[12] Tauber and Kleiner, *J. Biol. Chem.* 99, 249 (1932).

[13] Kleiner and Tauber, *J. Biol. Chem.* 100, 749 (1933).

[14] Fischl, *Chem. Ztg.* 57, 393 (1933).

a faintly alkaline copper and Rochelle salt solution containing phosphate, the reduction effected by sugars other than levulose is negligible in amount. As little as 1 mg. of levulose in the presence of 49 mg. of dextrose is thus detectable and determinable.

Reagents: 1) Copper ketose reagent: 15 g. of anhydrous sodium carbonate 5 g. of crystallized copper sulfate, 300 g. of Rochelle salt and 100 g. of disodium phosphate ($+ 12\,H_2O$) are dissolved in water in a 1 liter measuring flask to form a volume of about 900 cc. Solution is carried out as far as possible at room temperature, and is completed on a water bath. The flask is left in the bath for an hour and, when cold, the liquid is made up to 1 liter. It is then mixed with two teaspoonsful of active charcoal, and filtered. It should be stored in a dark glass bottle with a glass or rubber stopper and should not be kept too long.

Method: The levulose solution to be tested should be neutralized and diluted to contain about 0.5 per cent sugar. If it has been cleared with lead salts, the lead must be removed from the filtrate by means of anhydrous sodium sulfate and not by alkali or sodium phosphate. Ten cc. of the sugar solution and 30 cc. of the copper solution are placed in a 150 cc. flask, which is fitted with a cork carrying an accurate thermometer and channelled at the edge to prevent development of pressure during the heating. The flask is then placed in a water bath at 70° C. and its contents are swirled gently until they reach 65° C. This temperature and the swirling are maintained for exactly 5 minutes, after which the liquid is cooled as rapidly as possible in cold water to room temperature. The appearance of a turbidity or a precipitate of cuprous oxide shows the presence of levulose.

For the determination, the thermometer is washed with 20 cc. of water and the washings are caught in the flask. About 15 cc. of *N* hydrochloric acid and then a few cc. of 0.01 *N* iodine solution is poured carefully down the wall of the flask, without mixing. The whole is then mixed and a further quantity of 0.01 *N* iodine solution is added so that an excess of iodine solution is present. Thirty to 60 cc. are usually required depending upon the amount of cuprous oxide formed. The flask is stoppered and the iodine allowed to act for exactly two minutes, with occasional shaking. After the addition of starch solution the mixture is back-titrated with 0.01 *N* sodium thiosulfate solution. Unless more than 0.5 cc. of iodine solution has been used up, the presence of levulose cannot be assumed, since 50 mg. of dextrose requires about 0.3 cc. of the iodine solution. This method allows of the determination of levulose not only in

mixtures of sugars but also in sweet wines, fruit juices, honey and the like and is suitable for physiological chemical purposes.

Micro-modification of the Fischl method: 0.5 cc. of the clarified and lead-free sugar filtrate is placed in a small test tube and 1 cc. of the copper ketose reagent is added. The test tube is then placed in a water bath and is kept in this bath for 5 to 6 minutes at 65° to 70° with occasional swirling. At the end of this period, the tube is cooled as rapidly as possible to room temperature. Add 1 cc. of Benedict's arsenotungstic reagent. Shake thoroughly. Wait a minute or two for any gas evolved to escape, dilute with 5 cc. of water and compare against a standard sugar solution treated exactly the same way, in a colorimeter.

The Benedict's [15] arsenotungstic complex reagent is prepared as follows: Dissolve 10 g. of sodium tungstate in 60 cc. of water. Add 5 g. of pure arsenic pentoxide and 2.5 cc. of 85 per cent phosphoric acid. Then add 2 cc. of hydrochloric acid. Boil for 20 minutes, adding water occasionally to make up for the loss of water due to evaporation. Cool, add 6 cc. of formalin, 4.5 cc. of hydrochloric acid and 4 g. of sodium chloride. Dissolve all the materials and dilute to 100 cc.

SUCROSE

Chemical Methods

Determine the reducing sugars, clarification having been effected with neutral lead acetate, never with basic lead acetate, as directed under the Munson and Walker method and calculate to invert sugar from Table 1 appendix. Invert the solution as directed in the section, "Sucrose by polarization before and after inversion with hydrochloric acid"; exactly neutralize the acid; and again determine the reducing sugars, but calculate them to invert sugar from the table referred to above, using the invert sugar column alone. Deduct the percentage of invert sugar obtained before the inversion from that obtained after inversion and multiply the difference by 0.95 to obtain the percentage of sucrose. The solutions should be diluted in both determinations so that no more than 240 mg. of invert sugar is present in the quantity taken for reduction. It is important that all the lead be removed from the solution with anhydrous powdered potassium oxalate or sodium carbonate before reduction.

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[15] Benedict, *J. Biol. Chem.* 68, 759 (1926).

Optical Methods

All sugars and carbohydrates have asymmetric carbon atoms and therefore exhibit the phenomenon of optical rotation. The polarimeter which was discussed in Chapter II is an instrument which measures the optical rotation of a solution. It was explained that the specific rotation is dependent on the concentration of the active substance in grams per 100 cc., the length of the tube and the observed rotation. Hence knowing the specific rotation of the sugar, with the same tube and an observed rotation, the concentration of an unknown sugar solution may be calculated.

This procedure is greatly simplified by the use of a saccharimeter which is a polarimeter having an arbitrary scale reading per cent sugar directly, provided a factor weight, such as 26 g./100 cc., is used. Concentration and temperature have a marked effect on the specific rotation of the various sugars. All of the sugars commonly met with in food analysis are dextrorotatory except levulose and invert sugar.

International Sugar Commission Rules

"In general all polarizations are to be made at 20° C."

"The verification of the saccharimeter must also be made at 20° C. For instruments using the Ventzke scale 26 g. of pure dry sucrose, weighed in air with brass weights, dissolved in water so that 100 cc. of solution is obtained at 20° C., and polarized in a room the temperature of which is also 20° C., must give a saccharimeter reading of exactly 100.00. The temperature of the sugar solution must be kept constant at 20° C. during the polarization."

"In determining the polarization use the whole normal weight for 100 cc. or a multiple thereof or any corresponding volume."

"As clarifying and decolorizing agents use basic lead acetate, alumina cream, or a concentrated solution of ammonia alum. Boneblack and decolorizing powders are to be excluded." The A. O. A. C. adds whenever reducing sugars are determined in the solution for polarizing, use only neutral lead acetate for clarification, as basic lead acetate causes precipitation of some of the reducing sugars.

"After bringing the solution exactly to the mark at the proper temperature, and after wiping out the neck of the flask with filter paper, pour all the well-shaken clarified sugar solution on a rapidly acting, dry

filter. Reject the first portion of the filtrate and use the remainder, which must be perfectly clear for polarization."

"Whenever white light is used in polarimetric determinations, the same must be filtered through a solution of potassium dichromate of such a concentration that the percentage content of the solution multiplied by the length of the column of the solution in centimeters is equal to nine." The A. O. A. C. adds that this concentration must be doubled in polarizing carbohydrate materials of high rotation dispersion, such as commercial glucose, etc.

The 26 g. of pure dry sucrose referred to above is known as the normal weight.

Conversion Factors of Different Saccharimeter Scales

1° Ventzke Sugar Scale	= 0.34657° Angular Rotation D
1° Angular Rotation D	= 2.88542° Ventzke Sugar Scale
Normal weight Ventzke Scale	= 26.026 g.
1° Bureau Standards Scale	= 0.34620° Angular Rotation D
1° Angular Rotation D	= 2.88850° Bureau of Standards Scale
Normal weight Bur. of Stand. Scale	= 26.000 g.
1° Bidecimal Scale	= 0.26622° Angular Rotation D
1° Angular Rotation D	= 3.75629° Bidecimal Scale
Normal weight Bidecimal Scale	= 20.000 g.
1° French Sugar Scale	= 0.21666° Angular Rotation D
1° Angular Rotation D	= 4.61553° French Sugar Scale
Normal weight French Scale	= 16.29 g.
1° Wild Sugar Scale	= 0.13284° Angular Rotation D
1° Angular Rotation D	= 7.52814° Wild Sugar Scale
Normal weight Wild Sugar Scale	= 10.00 g.

The designation D refers to sodium light of 5893° Ångström.

Sucrose by Polarization Before and After Inversion with Hydrochloric Acid

Weigh double the normal weight of the material to be examined, that is 52 g. exactly and as rapidly as possible transfer the material to a 200 cc. sugar volumetric flask with water. Add a sufficient amount of

clarifying agent avoiding any excess. Shake, dilute to the mark with water, mix thoroughly and filter. Keep the funnel covered during the filtration and reject the first 25 cc. of the filtrate. If a lead clarifying agent was used, remove the lead from the filtrate by the addition of small portions of anhydrous potassium oxalate, anhydrous sodium carbonate and sometimes anhydrous potassium sulfate. Avoid any excess of the deleading agent, mix thoroughly and filter through a dry filter rejecting the first 25 cc. of the filtrate. Pipette 50 cc. of the lead free filtrate into a 100 cc. volumetric flask, dilute with water to the mark and polarize in a 200 mm. tube. The result multiplied by 2 is the direct reading.

Pipette another 50 cc. portion of the lead free filtrate into a 100 cc. volumetric flask and add 25 cc. of water. Add in small portions while rotating the flask, 10 cc. of hydrochloric acid (sp. gr. 1.1029). Adjust a constant temperature bath at 70° C. Place the flask containing a thermometer in the bath and when the thermometer reads 67° C., leave the flask in the bath for exactly 5 minutes longer. At the end of this period remove the flask and place it immediately in a bath at 20° C. When the contents of the flask reach 35° C., remove the thermometer from the flask, rinse it and fill almost to the mark. Allow the flask to remain in the bath for another half hour and then make to volume. Mix well and polarize the solution in a 200 mm. tube at 20° C. This reading must also be multiplied by 2 to give the invert reading. Calculate sucrose by use of the following formula.

$$S = \frac{100\ (P - I)}{143 + 0.0676\ (m - 13) - t/2} \qquad [1]$$

in which

S = the percentage of sucrose
P = direct reading, normal solution
I = invert reading, normal solution
t = temperature at which readings are made
m = g. of total solids in 100 cc. of the invert solution read in the polariscope.

The total solids are determined by observing the refractometer reading of the invert solution read in the polariscope and referring to Table 3, appendix, of sugar solids. The percent total solids is multiplied by the density corresponding to this percentage in the same table. The result is the grams of total solids in 100 cc. of the invert solution.

The inversion may be made at room temperature by the following procedures. 1) To 50 cc. of the lead free filtrate in a flask add 10 cc. of the above mentioned hydrochloric acid and set aside for 24 hours at a temperature not below 20° C.; or 2) if the temperature is above 25° C., set aside for 10 hours. Make up to volume of 100 cc. at 20° C. and polarize as directed above. The above formula must be corrected by changing the factor 143 to 143.2.

Sucrose by the Double Dilution Method

As was mentioned previously in the chapter, one of the errors in determining sugars, if clarifying agents are used, is the volume of the precipitate formed by the clarifier. If the volume of the precipitate is large, more than 1 cc. from 26 g. of material, then the Clerget or the double dilution method may be used to correct this error.

Weigh a half-normal weight of the sample and transfer it with the aid of water to a 100 cc. sugar volumetric flask. Clarify with the appropriate agent and make to volume. At the same time weigh a normal weight of the sample, transfer this material to another 100 cc. sugar volumetric flask, add the same clarifier and dilute to volume. Filter, and obtain the direct readings as directed above. Invert each solution by one of the methods detailed above and obtain the invert readings.

The true direct polarization of the sample equals 4 times the direct polarization of the diluted solution less the direct polarization of the undiluted solution. The true invert polarization equals 4 times the invert polarization of the diluted solution less the invert polarization of the undiluted solution. Calculate the sucrose from the true polarizations thus obtained, using the formula given above.

COMMERCIAL GLUCOSE

As was mentioned before commercial glucose is not to be confused with dextrose. Because of its method of manufacture commercial glucose is of variable composition and contains, dextrose, dextrins and maltose. Commercial glucose may be detected in food products by the following qualitative test developed by Fiehe.[16] Dilute or dissolve 10 g. of the sample with 10 cc. of water, warming if necessary to effect solution. Add 10 cc. of a saturated solution of ammonium oxalate and boil. Add animal charcoal and boil again. Filter and place 2 cc. of the clear filtrate in a

[16] Fiehe, *Z. Nahr. Genussm.* 8, 30 (1909).

test tube, add 2 drops of hydrochloric acid and 20 cc. of 95 per cent alcohol and mix. In the presence of commercial glucose or dextrins a marked white turbidity will be formed. This turbidity is due to the precipitated dextrins.

Another test for commercial glucose and better than the Fiehe test for products containing much protein material is the test devised by Jacobs and detailed in Chapter VII in the malted milk section.

The A. O. A. C. gives the following two methods for the estimation of commercial glucose: *Method I*, for substances containing little or no invert sugar: Commercial glucose cannot be determined accurately owing to the varying quantities of dextrin, maltose and dextrose present in the product. However in syrups in which the quantity of invert sugar is so small as not to affect appreciably the result, commercial glucose may be estimated approximately by the following formula:

$$G = \frac{(a - S)\ 100}{211}$$

in which

G = percentage of commercial glucose solids
a = direct polarization, normal solution
S = percentage of sucrose

Express the results in terms of commercial glucose solids polarizing +211° V.

Method 2, for substances containing invert sugar: Prepare an inverted half-normal solution of the substance as directed in the section "Sucrose by Polarization before and after Inversion with Hydrochloric Acid," by the rapid method, except cool the solution after inversion, make neutral to phenolphthalein with sodium hydroxide solution, slightly acidify with hydrochloric acid (1 : 5) and treat with 5–10 cc. of alumina cream before making up to the mark. Filter and polarize at 87° C. in a 200 mm. jacketed metal tube, preferably silver. Multiply the reading by 200 and divide by the factor 196 to obtain the quantity of commercial glucose solids polarizing +211° V.

The specific rotation of levulose decreases with increasing temperature until at 87° C. it just equals that of the dextrorotatory dextrose, hence in a solution of sugars which has been inverted containing commercial glucose, the commercial glucose will be the only polarizing sugar. The temperature at which exact neutralization occurs varies somewhat with the concentration of the mixture but 87° C. is the accepted temperature.

Chemical Methods for Sugars Other Than Sucrose

Dextrose, levulose, maltose and lactose are generally determined chemically as reducing sugars by their action on alkaline copper tartrate solution. The details are followed exactly as described in the Munson and Walker and Lane-Eynon methods and the quantity of copper found is referred to the proper factors and columns in Tables 1 and 2, appendix.

HONEY

Honey is the nectar and saccharine exudations of plants gathered, modified and stored in the comb by honeybees. Honey is laevorotatory and should not contain more than 25 per cent water, 0.25 per cent ash and 8 per cent of sucrose. The essential constituents of honey are dextrose, levulose and sucrose in small amounts together with lesser quantities of mineral matter, proteins, wax, pollen and sometimes mannitol and dextrins. The composition varies with the feeding of the bees. The presence of more than 8 per cent of sucrose in honey indicates that the bees have been fed on cane sugar and that the honey is not matured or that it is adulterated. In general the invert sugar content is high, circa 60 per cent and as the definition indicates honey is laevorotatory before and after inversion. The composition of typical honey is tabulated in Tables 14 and 15.

Table 14. Honey, Levorotatory [17] (United States)

	Maximum	Minimum	Average
Polarizations (°V) Direct			
Immediate	—21.90	+ 3.70	—11.24
Constant	—24.80	— 0.30	—14.73
Birotation	11.60	1.40	3.49
at 87°C	+23.70	+ 0.50	+10.15
Invert			
at 20°C	—29.26	— 1.32	—19.16
at 87°C	+23.21	+ 0.66	+ 7.91
Difference	33.55	23.32	27.07
Complete Analysis			
Water %	26.88	12.42	17.70
Invert Sugar %	83.36	62.23	74.98
Sucrose %	10.01	0.00	1.90
Ash %	0.90	0.03	0.18
Dextrin %	7.58	0.04	1.51
Undetermined %	7.45	0.04	3.73
Free Acid as Formic %	0.25	0.04	0.08

[17] Bryan, U. S. Dept. Agr., Bur. Chem., Bull. No. 154 (1912).

TABLE 15. HONEY[18] (CUBAN, MEXICAN, HAITIAN)

	Maximum	Minimum	Average
Polarizations (°V) Direct			
Immediate	—22.90	— 6.05	—13.34
Constant	—24.15	— 8.50	—14.52
Birotation	3.55	0.00	1.18
at 87°C	+17.00	+ 3.20	+10.31
Invert			
at 20°C	—26.07	— 8.86	—16.22
at 87°C	+15.40	+ 2.86	+ 9.08
Difference	28.93	22.77	25.30
Complete Analysis			
Water %	27.00	16.05	21.26
Invert Sugar %	77.56	68.09	72.38
Sucrose %	3.98	0.00	0.80
Ash %	0.58	0.06	0.21
Dextrin %	3.96	0.26	1.24
Undetermined %	8.07	0.66	4.11
Free Acid as Formic %	0.43	0.00	0.15

PREPARATION OF SAMPLE

If the sample is clear and appears to be homogeneous, mix thoroughly and it is ready for analysis. If part of the material has crystallized out, the entire sample should be warmed to about 40° C. and stirred until the crystals dissolve and if parts of the comb or other foreign material is present the mixture should be strained through cheese cloth or strained by some similar method.

Total solids, ash, soluble ash, alkalinity of the ash, color, acidity, metals and preservatives may be determined and detected in the usual manner.

POLARIZATION

Weigh 26 g. of the sample, the normal weight and transfer to a 100 cc. sugar volumetric flask with water. Add 5 cc. of alumina cream for clarification purposes, make to the mark at 20° C. and filter. Discard the first portion and polarize in a 200 mm. tube at the customary temperature. This is called the immediate direct polarization. Allow to stand overnight or add anhydrous sodium carbonate until distinctly alkaline and again polarize. This is termed the constant direct polarization. The difference between the immediate direct polarization and con-

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18 Bryan, U. S. Dept. Agr., Bur. Chem., Bull. No. 154 (1912).

stant direct polarization is the measure of the mutarotation. Polarize again at 87° C. to obtain the direct polarization at 87°.

Invert 50 cc. of the filtrate as directed in the section on inversion of sucrose and polarize at 20° and at 87° C. to obtain the corresponding invert polarizations.

Reducing Sugars

Reducing sugars may be determined before inversion by either the Lane-Eynon method or by the Munson-Walker method. Ten cc. of the filtrate from the direct polarization determination is diluted to 250 cc. and 25 cc. of this solution may be taken for the estimation, in the case of the Munson and Walker method. The result is calculated to percentage invert sugar with Table 1, appendix.

Reducing sugars after inversion may be determined by diluting 10 cc. of the solution used for the invert polarization with water, neutralizing with sodium carbonate and then diluting to 250 cc. Fifty cc. of this solution is used for the determination of reducing sugars by the Munson and Walker method.

Calculation of Sugar Percentages

Sucrose may be calculated from the polarization results by means of formula [1]. Rather better results are obtained by calculating sucrose from the reducing sugar determinations because of the error due to the change in optical rotation of levulose before and after inversion.

% invert sugar after inversion − % invert sugar before inversion $\times 0.95 =$ % sucrose.

Because of the large amount of levulose and its unequality to the amount of dextrose present, the polarization is not null as would be true in the case of inverted sucrose. Furthermore because of this reason, commercial glucose cannot be detected by the 87° C. polarization method. The percentage levulose may be calculated as follows:

$$\frac{a - 1.0315\ b}{2.3919} = L$$

where $a =$ constant direct polarization at 20° C.
$b =$ direct polarization at 87° C.
$L =$ g. of levulose in normal weight of honey (26 g.)

and $L/26 \times 100 =$ % levulose.

Levulose may also be estimated by the Fischl method.

The percentage of dextrose is equivalent to the percentage of invert sugar less the percentage of levulose.

Commercial Glucose

Since pure honey contains dextrins, no conclusion can be derived from the mere presence of dextrins. However, in the case of honey the dextrins have been hydrolyzed to such a degree that erythrodextrin is not a normal constituent. On the other hand in the case of commercial glucose, the hydrolysis seldom goes so far that all of the erythrodextrin is gone. This is the basis for the detection of commercial glucose in honey. Dilute some honey with an equal portion of water and add a few cc. of a solution of 1 g. of iodine and 3 g. of potassium iodide in 50 cc. of water. Run a control with a known sample of honey, adding the same amount of water and reagent. The production of a red or violet color indicates the presence of commercial glucose. If the honey is colored very deeply so that the red or violet color may be obscured, the dextrins may be precipitated by the addition of alcohol and after allowing to stand, the dextrins are separated by filtration, dissolved in water and the iodine solution added. The absence of color does not conclusively prove the absence of commercial glucose.

Dextrin

Transfer an accurately weighed portion of from 4–8 g. of honey with the aid of a Mojonnier pipette and carriage to a 100 cc. volumetric flask. Add 4 cc. of water, 1 cc. at a time and shake so that the honey dissolves and then fill to the mark with absolute alcohol, shaking vigorously during the addition of the alcohol. Allow the precipitate to settle completely and then filter by decantation. Wash with 10 cc. of 95 per cent alcohol, passing the washings through the flask. Dissolve the dextrins in the flask with several portions of hot water, passing the water through the filter and catching the filtrate in a tared dish. Evaporate to a small volume and dry to constant weight in vacuo. Determine the reducing sugars before and after inversion. Calculate the weight of the invert sugar before inversion and the sucrose and subtract from the weight obtained by drying the filtrate in vacuo. This gives the weight of dextrin and dividing by the weight of the original sample taken gives the percentage dextrin. This is an approximate determination.

Commercial Invert Sugar

In the manufacture of invert sugar commercially the invert sugar produced is contaminated with a small amount of furfural or its derivatives. The detection of these substances form the basis for the test for presence of commercial invert sugar in honey.

Transfer 5 cc. of honey to a test tube and add an equal amount of water. Add 5 cc. of ether and shake gently. Allow the layers to separate. Transfer 2 cc. of the clear ether layer to another test tube and then add 1 drop of a solution of 1 g. resorcinol in 100 cc. of hydrochloric acid. The production of an orange red to dark red color indicates the presence of commercial invert sugar.[19] Yellow to salmon pink colors are to be disregarded.

Another test may be made as follows: Mix 5 cc. of honey with 2.5 cc. of a mixture of 10 cc. of aniline and 3 cc. of 25 per cent hydrochloric acid. The immediate production of a bright red color indicates the presence of commercial invert sugar.[20]

Diastase

Pure honey if not heated to a temperature high enough to destroy it, contains the enzyme, diastase. The A. O. A. C. directs that it be determined as follows: Mix 1 part of honey with 2 parts of sterile water. Treat 10 cc. of this solution with 1 cc. of 1 per cent soluble starch solution and digest at 45° C. for an hour. At the end of this time test the mixture with 1 cc. of iodine solution (1 g. of iodine, 2 g. of potassium iodide, 300 cc. of water). Treat another 10 cc. portion of the honey solution, mixed with 1 cc. of the soluble starch solution without heating to 45° with the reagent and compare the colors produced. If the original honey has not been heated sufficiently to destroy the diastase, an olive-green or brown coloration will be produced in the mixture that has been heated at 45°. Heated or artificial honey becomes blue.

CONFECTIONERY

The many products that fall into this classification have to be analyzed according to the materials making the product. Thus chocolate confectionery and candy should be analyzed according to the methods for cocoa products. Sugared fruits may be treated as marmalade and jams. If

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19 Fiehe, *Z. angew. Chem.* 21, 2315 (1908).

20 Browne, U. S. Dept. Agr., Bur. Chem., Bull. No. 110 rev. (1916).

the confectionery is composed of different portions, these portions may be separated and analyzed by those methods applicable to those portions. In general mix or grind the product so as to obtain a homogeneous mass. On this well mixed sample the usual determinations of moisture or total solids, ash, soluble ash, alkalinity of the ash, metals, color, acidity, preservatives and nitrogen may be performed as described in other sections of the text. Sucrose may be estimated by polarimetric methods on a normal weight of the sample or by determining reducing sugars before and after inversion. The usual test for commercial glucose may be made. Fat or total ether extract may be determined by the introduction of 4–5 g. of the sample into a Mojonnier extraction tube or Jacobs-Singer separatory flask and then proceeding with the Roese-Gottlieb method exactly as described for the determination of fat in ice cream.

Paraffin

Saponify the extract in the fat flask obtained in the fat determination by the Roese-Gottlieb method by the addition of 10 cc. of 95 per cent alcohol and 2 cc. of sodium hydroxide solution (1 : 1). Heat on a steam bath under a reflux condenser until saponification is complete. Remove the condenser and evaporate off the alcohol. Transfer the material in the flask to a separatory funnel with the aid of hot water and extract when cool with 4 successive portions of petroleum ether. Collect the ether layers in a tared flask and evaporate to dryness. Place in an oven and dry to constant weight. An error is introduced due to the inclusion of the normal unsaponifiable matter present in the oil or fat used to make the confectionery but this would be so small as to be negligible. Any appreciable unsaponifiable matter would indicate the presence of paraffin.

Alcohol

Break the candy in such a manner as to collect the syrup and weigh out 30–50 g. of the syrup. Transfer with the aid of 40 cc. water to a distillation flask or better a Florence flask connected with a vertical condenser by means of a Polenske trap. Distill over nearly 50 cc., catching the distillate in a 50 cc. volumetric flask. Make to volume and determine the specific gravity with a pyknometer. Estimate the percentage of alcohol by reference to the alcohol table, Table 4, appendix.

Shellac

A simple qualitative test for the presence of shellac on candy is the following. Steep 25 g. of the candy in 50 cc. of 95 per cent alcohol overnight or warm on a steam bath for an hour or so. Decant the alcoholic extract into another beaker and evaporate down to a volume of about 5 cc. Bring 100 cc. of water to boiling in another beaker and pour the remaining alcoholic extract into the boiling water. If shellac is present, it will clump and rise to the surface of the boiling water. This is not done by other resins that may be extracted from candy such as chocolate.

MAPLE PRODUCTS

Maple syrup is the product obtained by the evaporation of the sap obtained by tapping the hard or rock maple. It may also be made by the solution of maple sugar. It should not contain more than 35 per cent water. Maple sugar is the solid product resulting from the evaporation of maple sap, or maple syrup. The syrup should be concentrated until it weighs 11 pounds to the gallon. This weight corresponds to a specific gravity of 1.325.

The principal constituent of maple syrup is sucrose. The syrup and the solution made from the sugar contain mineral matter, proteins, organic acids and flavoring materials which give it its characteristic flavor and odor. As in the case of fats and oils and as in jams and jellies, maple products have certain constants that are characteristic. The constants which are most indicative of the purity of these materials are according to Snell and Scott [21] refractometer reading, conductivity value, total ash, alkalinity of the ash, Winton lead number and the Canadian lead number. Ratios set up on the basis of these constants aid in the interpretation of the results.

Preparation of Sample

Because of the nature of the determinations, the samples of maple products must be prepared according to rigid specifications. The A. O. A. C. gives these official details for maple syrup. 1. *For solids determination.*—If the sample contains no sugar crystals or suspended matter, decant sufficient of the clear syrup for use in the determination. If sugar crystals are present, redissolve them by heating. If suspended matter is present, filter the sample through cotton wool.

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[21] Snell and Scott, *Ind. Eng. Chem.* 6, 219 (1914).

2) *For other determinations.*—If sugar crystals are present, redissolve them by heating. If other sediment is present, distribute it evenly through the syrup by shaking. Transfer approximately 100 cc. of the syrup, with its suspended sediment, to a casserole or beaker, add ¼ the volume of water, and evaporate over a flame. When the temperature of the boiling syrup approaches 104° C., draw a small quantity into a thin-walled pipette of about 1 cc. capacity and cool to room temperature, in running water. Wipe the outside of the pipette, allow the possible diluted syrup in the point to escape and make a refractometric measurement of the solids content of the cooled syrup. Repeat the procedure from time to time until a reading is obtained corresponding to 64.5 per cent solids ($n_{20} = 1.4521$), or to such other value as in the experience of the analyst will give a filtered syrup of 65.0 per cent solids. Filter the syrup through a filter which will allow the 100 cc. to pass within 5 minutes and adjust the filtrate to 65.0 ± 0.5 per cent solids (refractrometric) by thorough mixing with the appropriate quantity of water.

For maple sugar and other solid or semi-solid products.

1) *For moisture and solids determination.*—Grind in a mortar, if necessary, and mix thoroughly.

2) *For other determinations.*—Prepare a syrup by dissolving approximately 100 g. of the sample in 150 cc. of hot water, boil until the temperature approaches 104° C. and complete the preparation of the resulting syrup as directed above, commencing at "draw a small quantity into a thin-walled pipette."

Using the sample prepared as directed the usual determinations may be made for ash, soluble and insoluble ash, alkalinity of the ash, polarization, and sugars before and after inversion. Commercial glucose may be detected as previously described.

Lead Number

When basic lead acetate is added to a maple syrup or to a solution of maple sugar, a precipitate is formed due to the insoluble salts of malic and other organic acids and also of sulfates, chlorides and proteins. On the other hand solutions of pure cane sugar do not yield a similar type of precipitate. The amount of lead remaining in solution after the addition of basic lead acetate solution to a maple syrup or solution of maple sugar is a measure of the amount of precipitable material. This is the basis of the following test for the estimation of the Winton lead number. The Canadian lead number is based on the weight of the lead

precipitate. Since they are empirical methods, the official directions of the A. O. A. C. are given.

Canadian Lead Number (Fowler Modification)—*Reagent:* Standard basic lead acetate solution.—Activate litharge by heating it to 650–670° C. for 2.5–3 hours in a muffle. The cooled product should be a lemon color. In a 500 cc. Erlenmeyer flask provided with a return condenser boil 80 g. of normal lead acetate crystals and 40 g. of the freshly activated litharge with 250 g. of water for 45 minutes. Cool, filter off any residue, and dilute with recently boiled water to a density of 1.25 at 20° C.

Determination: Weigh the quantity of syrup containing 25 g. of dry matter, transfer to a 100 cc. flask, and make up to mark at 20° C. Pipette 20 cc. into a large test tube, add 2 cc. of the standard basic lead acetate solution, cork, and allow to stand 2 hours.

Filter with suction on a 25 cc. tared Gooch, having an asbestos mat at least 3 mm. thick. When nearly all the liquid has run through, fill the crucible with cold water. Repeat to a total of 4 washings, taking care to prevent formation of fissures in the precipitate by keeping it covered with water and avoiding too great suction. Dry at 100° C., weigh, and multiply the weight by 20.

Winton Lead Number—*Reagent:* Standard basic lead acetate solution.—To a measured volume of the reagent prepared for the determination of the Canadian lead number, add 4 volumes of water and filter. A blank should be run with each set of determinations.

Determination of lead in the blank: Transfer 25 cc. of the standard basic lead acetate solution to a 100 cc. flask, add a few drops of glacial acetic acid, and make up to the mark with water. Shake and determine lead sulfate in 10 cc. of the solution as directed immediately below. The use of acetic acid is imperative in order to retain all the lead in solution when the reagent is diluted with water.

Determination: Transfer 25 g. of the sample to a 100 cc. flask by means of water. Add 25 cc. of the standard basic lead acetate solution and shake. Fill to the mark, shake, and allow to stand for at least 3 hours before filtering. Pipette 10 cc. of the clear filtrate into a 250 cc. beaker, add 40 cc. of water and 1 cc. of sulfuric acid, shake, and add 100 cc. of 95 per cent alcohol. Allow to stand overnight, filter on a weighed Gooch crucible, wash with 95 per cent alcohol, dry in a water oven, and ignite in a muffle or over a Bunsen burner, applying the heat gradually at first

and avoiding a reducing flame. Cool, and weigh. Subtract the weight of lead sulfate so found from the weight of lead sulfate found in the blank, and multiply by the factor 27.33. The use of this factor gives the lead number directly without the various calculations otherwise required.

Sy Lead Number—A simple method based on the volume of the precipitate produced on the addition of the standard basic lead acetate solution is that developed by Sy.[22] In a 25 cc. graduated cylinder introduce 5 cc. of syrup or 5 g. of sugar which is afterwards dissolved in a little water. Add water to the 15 cc. mark and 2 cc. of the basic lead acetate solution. Shake thoroughly and allow the mixture to stand for 20 hours. Then read the volume of the precipitate, which for pure maple products should be at least 3 cc. and is usually over 5 cc. This method is not as accurate as the preceding methods.

Cowles [23] Malic Acid Value

Weigh 6.7 g. of the sample into a 250 cc. beaker; add 5 cc. of water, then 2 cc. of a 10 per cent calcium acetate solution; and stir. Add, gradually and with constant stirring, 100 cc. of 95 per cent alcohol and agitate the solution until the precipitate settles, or let stand until the supernatant liquid is clear. Filter off the precipitate and wash with 75 cc. of alcohol, 85 per cent by volume. Dry the filter paper and ignite in a platinum dish. Add 10 cc. of 0.1 *N* hydrochloric acid, and warm gently until all the lime dissolves. Cool, and titrate back with 0.1 *N* sodium hydroxide solution, using methyl orange indicator. The difference in cc. divided by 10 represents the malic acid value of the sample. Previous to use, the reagents should be tested by a blank determination and any necessary corrections applied.

Rapid Conductivity Method

The detection of adulterated maple syrup may be rapidly and easily performed by a determination of the conductivity of the syrup.[24] Because of the varying nature of maple products, it is impossible to define the two limits between which the conductivity values will fall. Conlin [25] on the

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22 Sy, *J. Am. Chem. Soc.* 30, 1430 (1908).
23 Cowles, *J. Am. Chem. Soc.* 30, 1285 (1908).
24 Snell, *Ind. Eng. Chem.* 5, 740 (1913).
25 Conlin, *Ind. Eng. Chem., Anal. Ed.* 7, 426 (1935).

basis of over 7,200 samples has adopted for the following method, primarily designed to detect adulteration with white sugar, a minimum value of 100 and values may range up to 200.

The apparatus consists of a Leeds and Northrup sugar ash bridge and conductivity cell, Fig. 50. No constant temperature bath is needed for a knob on the bridge provides for variations in temperature from 10–35° C. and variations in cell constant from 0.14 to 0.16 and all results are automatically corrected to 20° C.

Fig. 50. Sugar Ash Bridge

(Courtesy of Leeds and Northrup)

Place 75 cc. of water in a 100 cc. graduated cylinder, add 25 cc. of syrup, and mix thoroughly. Rinse off the electrodes with approximately 40 cc. of this solution, pour the remainder into the testing cylinder, and insert the electrodes and thermometer. Adjust the temperature compensating knob on the bridge to correspond with the cell reading, place the resistance plug in the proper setting (usually the 1000 ohm block), press the alternating current button, and turn the main slide-wire knob until the galvanometer balances. Take the dial reading. The product of this value and the plug setting is the specific conductance multiplied by 10^6 and reduced to 20° C. Since the conductivity value is defined by 10^5, the product is 10 times the desired figure.

Measurements at 25° C., which is the temperature required for the official A. O. A. C. method, average about 13 per cent higher than those made at 20° C. The average conductivity value of Canadian syrups is higher than that of American syrups. It is best to confirm doubtful values by the other methods given.

Interpretation of Results, Maple Products

Of all the various analyses for maple products, those used most frequently in determining the purity of a sample with respect to adulteration with other sugars, are: total ash, soluble and insoluble ash, malic acid value, Canadian or Winton lead number and conductivity.

There is more or less disagreement about the interpretation of the analytical data found by the above methods. Maple products, being unrefined, are subject to wide variations in their non-sugar content. It is upon this non-sugar content, which consists principally of the potassium and calcium salts of malic acid and smaller amounts of similar organic acids, that the analytical data depend. For example, one sample of pure maple sugar or syrup might have a total ash content of 1.20 per cent (calculated to a dry basis), while another, equally pure might have a total ash content of only 0.80 per cent. It is therefore obvious that, in judging an unknown sample, the minimum figures found in samples known to be pure must be used for comparison. It is also clear that samples showing high analytical data can at times be adulterated considerably without detection.

In Table 16 are tabulated the analyses of typical maple sugars and syrups. For the purpose of the discussion the minimum Vermont figures established by Jones [26] are given: Total Ash = 0.77 per cent, Insoluble Ash = 0.23 per cent, Malic Acid Value = 0.61. These figures are calculated on a moisture free basis. No definite minimum figures have been agreed on for lead numbers and none have been adopted for conductivity value, which is relatively new. (See conductivity section.)

In general, Canadian minimum figures are lower than those of Vermont, and light colored, mild flavored syrups will give lower analytical data than the darker strong syrups. There are, of course, frequent exceptions but this is always true when composite samples representing very large amounts of syrup are used. Below is a representative analysis of maple sugar made from a mixture of all grades of syrup.[27] It is an

[26] Jones, Vt. Agr. Exptl. Sta., 17th and 18th Annual Repts. (1904-5).

[27] Conlin, Private Communication (1937).

Table 16. Maxima, Minima and Average of Analyses of United States and Canada
Maple Sugar and Syrups[28,29]

	Average (363)	Maximum	Minimum
Sucrose, Clerget %	90.69	98.62	57.04
Invert Sugar %	6.19	35.26	0.00
Undetermined %	2.14	8.18	0.00
Total Ash %	0.98	1.70	0.76
Soluble Ash %	0.62	1.14	0.30
Insoluble Ash %	0.36	1.00	0.21
Soluble Ash/Insoluble Ash	1.69	4.07	0.43
Lead Number Winton	3.50	5.90	2.20
Malic Acid Value	0.93	1.72	0.51
Alkalinity			
Soluble Ash, cc.	75	140	42
Insoluble Ash, cc.	87	190	31
Soluble Ash/Insoluble Ash	0.86	2.29	0.37

average analysis representing 18,000 gallons of syrup. The data is calculated to a moisture free basis:

Total Ash	0.935%
Soluble Ash	0.572%
Insoluble Ash	0.363%
Alkalinity of Sol. Ash	7.57 cc.
Alkalinity of Insol. Ash	9.77 cc.
Malic Acid Value	0.883
Canadian Lead Number	3.71
Conductivity at 20° C.	124.

REFERENCES

Browne, "Sugar Analysis," Wiley (1912).
Woodman, "Food Analysis," McGraw-Hill (1931).
Leach-Winton, "Food Inspection and Analysis," Wiley (1920).
Methods of A. O. A. C. (1935).
Hawk and Bergeim, "Physiological Chemistry," Blakiston (1927).
Allen's Commercial Organic Analysis," 5th ed., Vol. I, Blakiston (1923).
Haworth, "Constitution of Sugars," Arnold (1929).
Armstrong and Armstrong, "Carbohydrates," Longmans (1934).

[28] Bryan, U. S. Dept. Agr., Bull. No. 466 (1917).
[29] Moisture free basis.

CHAPTER X

GUMS, CEREALS, STARCH AND OTHER POLYSACCHARIDES

GUMS

It was mentioned in Chapter VII that the use of binders and fillers has increased in recent years. One group of these substances is the class of gums. These are amorphous, transparent or translucent substances of wide distribution among plants, which form sticky masses with water and are insoluble in alcohol, acetone, ether, petroleum ether and, in general, organic solvents. They are generally odorless and tasteless. Some yield clear solutions with water and are completely filterable, whereas others swell in water and are only partially soluble and filterable. The first are called real gums and the second are termed vegetable mucilages. Gums are composed of hydrogen, oxygen and carbon and yield carbohydrate hydrolytic products. Hence, they are generally classified with the carbohydrates.

The gums may be grouped according to their origin as follows:

Plant and Tree Exudates

Acacia (arabic)
Ghatti (British Indian gum)
Karaya (Sterculia Indian gum)
Tragacanth (bassorin)

Sea Weed Extracts

Agar agar
Irish moss (chondrus crispus)

Seed Extracts

Locust kernel
Locust bean
Quince seed

Fruit and Vegetable Extracts

Pectins.

Acacia, ghatti, Irish moss, quince seed and the various pectins are readily soluble in water and are filterable. Karaya, tragacanth, agar, locust kernel and locust bean are the type that swell in water and are only partially soluble. Agar is difficultly soluble in the cold, but dissolves in boiling water and on cooling sets to a gel. Locust kernel is purified locust bean.

Acacia

Acacia, arabic is the dried gummy exudation from the stems and branches of *Acacia Senegal*, Wildenow, or of some other species of acacia. It forms tears of various sizes, whitish, yellowish white or light amber in color. Gum arabic is composed essentially, according to O'Sullivan,[1] of the calcium salt of arabin or arabic acid. Complete hydrolysis yields arabinose, galactose and arabinosic acid. Norman [2] confirmed the presence of arabinose and galactose as hydrolysis products and concluded that gum arabic has no definite formula, but probably consists of a nucleus acid made up of galactose and a uronic acid, probably, galacturonic acid to which is linked arabinose by glucosidic bonds, so that arabinose is more easily split off than the other components. Arabic contains an oxidase. The composition of acacia, according to Norman [2] is the following:

Ash %	0.24
Furfural yield (ash free) %	13.93
Carbon dioxide yield (ash free) %	4.39
Uronic acid anhydride	17.56
Furfural due to uronic acid %	2.91
Arabinose %	23.52
Galactose %	68.80

Agar

Agar agar is the dried mucilaginous substance extracted from marine algae or sea weeds, particularly, *Gelidium corneum* (Hudson) Lamouroux. It generally occurs in thin transparent membranes which are difficultly soluble in cold water but readily soluble in hot water. It contains diatoms which may be identified microscopically. The sulfate radical appears to be an integral part of the complex agar agar molecule and can only be

[1] O'Sullivan, *J. Chem. Soc.* 79, 1164 (1901).

[2] Norman, *Biochem. J.* 23, 524 (1929).

freed by hydrolysis with acid.[3] Agar is used extensively for making culture media in bacteriology.

Ghatti

Ghatti gum, which is also known as British Indian gum is a gummy exudation from the stem of *Anogeissus latifolia* Wall. It comes from India and Ceylon. Because it is called Indian gum sometimes, it must be carefully differentiated from karaya or sterculia gum. It forms yellowish white tears that are completely soluble in water and since this property is very similar to that of acacia, it is used sometimes to adulterate that gum. Tests for its distinction from arabic are detailed in the text.

Irish Moss

Irish moss is the dried, sunbleached plant of *Chrondrus Crispus* (Linne) Stackhouse. It is a sea weed or kelp and is found on the beaches of Ireland and Massachusetts. It is sometimes adulterated by bleaching with sulfur dioxide. The gum, termed carrageenin or algin, is extracted from the sea weed by boiling in water and is precipitated from the resultant mucilage by alcohol or some other precipitant.

The composition of the dried sea weed is given by McCance, Widdowson and Shackleton [4] as follows:

Water, g. per 100 g.	13.9
Unavailable carbohydrate (roughage), g. per 100 g.	71.3
Titratable acidity, cc. 0.1 *N* alkali per 100 g.	8
Reducing sugar, g. per 100 g.	0.0
Sucrose, g. per 100 g.	0.4
Starch, as glucose	0.0
Total available carbohydrate, g. per 100 g., as glucose	0.4
Total nitrogen, g. per 100 g.	1.08
Protein, g. per 100 g.	6.8

Karaya

Karaya, also known as Indian tragacanth gum or sterculia gum, is an exudate of the trees belonging to the genus *Sterculia urens* Roxb., found

[3] Parkes, *Analyst* 46, 239 (1921).

[4] McCance, Widdowson and Shackleton, Med. Research Council, Special Rep. Ser., No. 213 (1936).

in India. Because of its similarity to tragacanth in swelling in water instead of dissolving, it is sometimes used to adulterate tragacanth. Karaya has a high volatile acidity number, varying from 13.4 to 21.3, expressed in terms of acetic acid.[5] On long standing the gum develops a distinct odor of acetic acid.

Locust Kernel

Locust kernel gum is a gum which has been produced on large commercial basis in recent years. It is the dried mucilaginous extract of locust bean, otherwise known as the carob-bean or St. John's bread. Locust kernel is differentiated from locust bean in that locust bean contains starch and is less pure, from the gum standard, than locust kernel. Knight and Dowsett [6] give as the composition of the commercial gum, the following:

Galactan %	29.18
Mannan %	58.42
Pentosans %	2.75
Protein %	5.29
Nitrogen %	0.83
Cellular tissue %	3.64
Ash %	0.82

The analysis shows that the commercial product is not a pure carbohydrate.

Quince Seed

This gum may be obtained by extraction from quince seeds. With warm water, the seeds swell up and form a mucilaginous mass. The mucilage is used in bandoline and cosmetic hair lotions. The mucilage is sometimes evaporated to dryness and the residue powdered. The powder may then be used to remake the mucilage. Quince seed is representative of a large group of seeds that yield mucilaginous extracts with water. Others in this group are psyllium seed and flax seed. Hanshe and Still [7]

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[5] Tschirch and Fluck, *Pharm. Acta Helv.* 3, 151 (1928).
[6] Knight and Dowsett, *Pharm. J.* 136, 35 (1936).
[7] Hanshe and Still, *Am. J. Pharm.* 105, 433 (1933).

give as the composition of the dried aqueous extract of the hulls of psyllium seed, the following:

Ash %	2.53
Total nitrogen %	0.13
Total phosphorus %	0.14
Pentose %	83.05

Again, it can be seen that the mucilage is not a pure carbohydrate.

Pectin

Pectins are a group of carbohydrate compounds which occur in fruits and vegetables. The pectin content increases with the ripening of the fruit. Pectin has no definite composition. Pectic acid [8] consists of polygalacturonic acids combined with varying numbers of molecules of *l*-arabinose and *d*-galactose. Bonner [9] suggests that pectic acid consists of chains of molecules of *d*-galacturonic acid interspersed with occasional molecules of *l*-arabinose and *d*-galactose. Pectic acid is presumed to be released from pectin by the action of enzymes and the pectic acid so formed forms a gel with calcium. This is one of the properties upon which the making of jams and preserves depends.

Ehrlich [10] describes a typical reaction given by *d*-galacturonic acid and hydrolyzed pectin, with basic lead acetate solution. If an aqueous solution of alpha or beta *d*-galacturonic acid is treated with a little freshly filtered basic lead acetate solution, a colorless flocculent precipitate is formed which redissolves as more of the reagent is added. The resulting clear solution, when heated in a water bath, becomes turbid and pink in a few seconds, the pink color of the rapidly increasing turbidity visibly changes to a deep red and after about 1 minute a dark blood-red to brick-red precipitate separates in thick flocks and the supernatant liquid becomes colorless or, in the presence of a large excess of lead acetate solution, yellowish. This amorphous red salt, which is formed by the decomposition of the original basic lead salt of *d*-galacturonic acid under the influence of the excess of lead acetate in hot solution, is also

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[8] Anderson, *J. Biol. Chem.* 121, 165 (1937).
[9] Bonner, *Bot. Rev.* 2, 475 (1936).
[10] Ehrlich, *Ber.* 65B, 352 (1932).

formed when only enough lead acetate is added to form a heavy white precipitate and the mixture, which should be distinctly alkaline to litmus, is heated 1 minute. In this way 5 mg. of *d*-galacturonic acid gives a distinct red precipitate and even 1 mg. can be faintly detected after the heated mixture has stood some hours. The soluble salts of *d*-galacturonic acid behave in a similar manner.

Tragacanth

Tragacanth is the dried gummy exudation from *Astragalus gummifer* Labillardiere, or other species of Astragalus. The soluble portion of this gum yields arabinose, galactose and geddic acid on hydrolysis.[11] The insoluble portion is called bassorin. Tragacanth generally contains a small amount of starch and consequently gives a blue color on the addition of iodine solution.

Norman [12] states that the soluble portion, which he calls tragacanthin, consists solely of uronic acid and arabinose. He was unable to verify the work of O'Sullivan as to the presence of galactose.

Reactions of the Gums

The composition of the gums has never been thoroughly investigated. In fact, some of the previously mentioned citations show that the commercial products are not pure carbohydrates. Furthermore, since many of the gums give common hydrolyzates, little purpose is gained by this type of analysis. Moreover, they are not crystalline bodies nor do they have the characteristic granular structure that starch grains have and possess, at times, even after incorporation into foods, therefore, they cannot easily be distinguished by microscopic means.[13] Jacobs and Jaffe [14] made an extensive survey of the reactions of the gums and showed that the gums differed in their reaction with various inorganic reagents.

Because of the scarcity of material on the subject of gums and the increasing use of these substances in foods, the reactions of the gums are detailed in Table 17. Table 18 gives the characteristic reactions for the identification of each gum.

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[11] O'Sullivan, *J. Chem. Soc.* **79**, 1164 (1901).

[12] Norman, *Biochem. J.* **25**, 200 (1931).

[13] Wildman, *J. Assoc. Official Agr. Chem.* **18**, 637 (1935).

[14] Jacobs and Jaffe, *Ind. Eng. Chem., Anal. Ed.* **3**, 210 (1931).

TABLE 17. RESULTS OF TESTS IN VARIOUS GUMS

Gum	Stokes Acid Mercuric Nitrate Reagent	Neutral Lead Acetate 20% Soln.	Basic Lead Acetate (A. O. A. C.)	Potassium Hydroxide 10% Soln.	Neutral Ferric Chloride 5% Soln.	Alcohol Precipitate	Borax, 4% Soln.	Millon's Reagent	+2 Vols. Acetic Acid
Arabic	White, fine, opaque ppt., sol. in excess of reagent	No ppt.	White, curdy ppt., insol. in excess	Faint yellow tinge	Ppt. sol. in excess	Very fine floc., non-adherent 40 cc. pt. of definite pptn.	Negative	Yellow, fine ppt.	Negative
Tragacanth	Voluminous flocculent translucent ppt.	Voluminous flocculent ppt., gels	Voluminous ppt., gels	Bright yellow, stringy ppt.	Gelatinizes	Coag. long and stringy, adherent, 10 cc.	Negative	Vol. floc. ppt.	Negative
Agar-agar	Gelatinizes	Flocculent ppt., gels	Voluminous ppt.	Clarifies soln.	Gelatinizes, heat + excess → ppt.	Heavy floc., adherent to beaker, 20 cc.	Negative	Fine ppt.	Ppt.
Karaya	White, curdy, ppt. settles rapidly	Negative	Stringy ppt., settles rapidly	Negative	Ppt. coag. on heating	Fine filamentous particles, non-adherent, 15-20 cc.	Negative	Fine ppt.	Negative
Irish moss	Gelatinizes	Flocculent ppt., gels	Voluminous flocculent ppt., gels	Gels	Vol., stringy ppt., gels	Coagulated, translucent, stringy, adherent, 20 cc.	Negative	Fine ppt.	Negative
Quince seed	Voluminous flocculent yellowish ppt.	Yellowish flocculent ppt., gels	Yellowish vol., flocculent ppt., gels	Stringy ppt.	Stringy ppt.	Coag., short, stringy, non-adherent, 25 cc.	Negative	Floc. ppt.	....
Locust kernel	Gelatinizes	Voluminous ppt., gels	Gels	Slight floc., ppt.	Stringy ppt.	Stringy, clotty, opaque, non-ad., start, 2 cc., complete, 15 cc.	Gels	Fine ppt.	Ppt.
Ghatti	No ppt.	No ppt.	Translucent floc. ppt.	Negative	Negative	Fine floc. ppt., non-adherent	Negative	Fine ppt.	Negative
Pectin	Floc. ppt., gelatinizes	Floc. ppt., gelatinizes	Floc. ppt., gelatinizes	Ppt.	Ppt.	Floc. ppt. translucent	Negative	Floc. ppt.	Ppt.
Starch	Negative	Negative	Negative	Negative	Negative	White turbidity ppt.	Negative	Fine ppt.	White ppt.
Dextrin	Negative	Negative	Negative	Negative	Negative	White turbidity	Negative	Fine ppt.	Negative

Table 18. Characteristic Reactions of Gums

Gum	Reaction
Arabic	Very soluble. It is not precipitated by neutral lead acetate solution. Gives a precipitate with 1 drop of 10% ferric chloride which is soluble in excess of ferric chloride.
Agar agar	An aqueous 0.2% solution forms a gel. Five cc. of an 0.1% solution + 1 drop sulfuric acid + 1 drop of congo red and centrifuged yields a flocculent precipitate.
Ghatti	Very soluble. Fresh solutions do not yield a curdy white precipitate with basic lead acetate, distinction from arabic.
Irish moss	Has a distinct odor of sea weed. It is precipitated by a saturated solution of barium chloride.
Karaya	Has a very high volatile acid number and gives a pink color when boiled with phosphoric, hydrochloric or trichloroacetic acid.
Locust kernel	The addition of 1 to 2 drops of 4% borax solution to 0.5% gum solution yields a gel. Tannic acid gives a precipitate.
Quince seed	Yields a precipitate with 1 or 2 drops of saturated zinc chloride solution or with ammonium molybdate reagent. Also gives a precipitate with saturated barium chloride solution but Irish moss does not give precipitates with the former reagents. Quince seed also gives a flocculent precipitate when treated as described under agar, but agar does not give the zinc chloride or ammonium molybdate test.
Tragacanth	Boiled with 10% potassium hydroxide solution yields a yellow stringy precipitate and a solution tinged with yellow.
Pectin	Gives a precipitate of digalacturonic acid when treated as described in the chapter on jams and jellies, Chapter XI. Quince seed may also give a precipitate but pectin does not give the zinc chloride or ammonium molybdate reactions.

These reactions are given by the gums in water solutions prepared from the gum as it is sold commercially and depend very often on the concentration of the gum. Thus 5 cc. of 0.5 per cent solution of locust kernel gum is jellied by a few drops of 4 per cent borax solution but an 0.2 per cent solution is not jellied by this reagent. Table 17 gives the reaction of solutions of the gums that vary in concentration from 0.5 per cent to 1 per cent, except for agar, for which 0.1 to 0.2 per cent solutions are used. In order to make use of the table properly, care must be taken to notice the distinguishing characteristics of the precipitate formed. That is, whether it is voluminous, flocculent, small flocculent, stringy, powdery, curdy, filamentous, etc. With certain reagents there is no apparent change, hence the use of blanks and controls is essential. The word "gel" in the table indicates an actual jellied condition. The word "gelatinizes" implies that the mixture has been thickened. These are clear distinctions and are readily noticeable in making the tests.

The **reagents** used are prepared as follows:

Stoke's reagent. Dissolve metallic mercury in twice its weight of nitric acid and dilute this solution to 25 times its volume with water.

Millon's reagent. Dissolve metallic mercury in an equal weight of nitric acid and dilute the solution with an equal volume of water.

Neutral Lead Acetate. Dissolve 20 g. of lead acetate, $Pb(C_2H_3O_2)_2 \cdot 3H_2O$ in water and make up to 100 cc.

Basic Lead Acetate. Boil 430 g. of neutral lead acetate, 130 g. of litharge, and 1 liter of water for 30 minutes. Allow the mixture to cool and settle and then dilute the supernatant liquid to a specific gravity of 1.25 with recently boiled water. Solid basic lead acetate may be substituted for the normal salt and litharge in the preparation of the solution. The solid salt is dissolved in water and diluted to the proper specific gravity.

Ammonium Molybdate Solution. Dissolve 100 g. of molybdic acid, MoO_3, in a mixture of 144 cc. of ammonium hydroxide and 271 cc. of water. Pour this solution slowly and with constant stirring into a mixture of 489 cc. of nitric acid and 1148 cc. of water. Keep the final mixture in a warm place for several days or until a portion heated to 40° C. deposits no yellow precipitate of ammonium phosphomolybdate. Decant from any sediment and preserve in glass stoppered vessels.

The **procedure** in general is to add 2 to 3 drops of the reagent to 5 cc. of the test solution, note the result and then add an excess of the reagent. In the case of some of the reagents namely, potassium hydroxide, phosphoric acid, hydrochloric acid and ferric chloride, a few drops of the reagent is added to the test solution and the mixture boiled. Then an excess of the reagent is added and the mixture is boiled again. For noting the type of alcohol precipitate, the technique of Weinberger and Jacobs [15] is recommended. To 20 cc. of the test solution, add 70 cc. of 95 per cent alcohol drop by drop, with constant stirring until precipitation is complete. Note the texture, quality and the characteristics of the precipitate, as well as the point at which definite precipitation begins.

Volatile Acidity

In various parts of this chapter it was indicated that karaya had a high acid number and hence could be differentiated from other gums. The following method may be used for estimating the volatile acidity

[15] Weinberger and Jacobs, *J. Am. Pharm. Assoc.* **18**, 34 (1929).

of gums. This value varies from 13.4 to 21.3 for sterculia gum and from 2 to 2.4 for gum tragacanth. The gum is placed in solution with phosphoric acid and is then steam distilled. The amount of acid is subsequently estimated in the distillate by titration with standard alkali.

Transfer about 1 g. of the sample accurately weighed to an 800 cc. Kjeldahl flask and allow the gum to soak in a mixture of 100 cc. of water and 5 cc. of syrupy phosphoric acid overnight or for several hours. Reflux cautiously for 2 hours. Attach the Kjeldahl flask to a steam distillation system and steam distill until 600 cc. of distillate have been obtained. Use a small boosting flame under the Kjeldahl flask and a trap or scrubber between the flask and the condenser. Do not allow the contents of the Kjeldahl flask to become so concentrated that the material chars. Titrate the distillate with 0.1 *N* sodium hydroxide solution using 10 drops of phenolphthalein indicator. Correct the determination by running a blank and express the result as the number of cc. of 0.1 *N* sodium hydroxide solution required to neutralize the volatile acidity obtained per g. The volatile acid is assumed to be acetic acid.

Detection of Gums in Food Products

In general, it is necessary for the food analyst to determine the presence of gums as a class rather than identify the gum itself. The following method lends itself to the detection and possible identification of gums in various food products.

Weigh 50 g. of the food material into a beaker and if necessary remove fatty materials by adding petroleum ether, stirring, and after allowing to settle, pouring off the supernatant petroleum ether layer. Repeat until practically all of the fat has been removed. Three or four washings with petroleum ether suffice. Evaporate off any residual petroleum ether on a steam bath. Add 50 cc. of water to the petroleum ether washed residue and stir vigorously until a paste is formed. Add 10 cc. of 40 per cent trichloroacetic acid and again stir vigorously. Allow to stand for 5 minutes. Transfer the pasty mixture to a centrifuge bottle and centrifuge for 5 to 10 minutes. Filter. Test a portion of the clear filtrate for complete precipitation of proteins with more trichloroacetic acid. If a precipitate develops, add more trichloroacetic acid and recentrifuge and filter. Add 5 volumes of 95 per cent alcohol to the filtrate with constant stirring and allow to stand for 5 minutes. Add ammonium hydroxide solution drop by drop until the mixture is alkaline and again allow to stand for 5 minutes. Any split-proteins not thrown out by the trichloro-

acetic acid will appear at this point as well as the gums. Add 10 drops of hydrochloric acid and stir vigorously. A flocculent or stringy precipitate at this time indicates the presence of gums, for the split-proteins or pseudo-gelatins will generally dissolve in the hydrochloric acid. Starches and dextrins behave as do the gums and do not dissolve.

Allow the mixture to stand for at least an hour or preferably overnight to permit the precipitate to settle and agglomerate. Filter the precipitate through a Gooch crucible equipped with a thin pad of asbestos. Wash the residue thoroughly in the crucible with alcohol. Dry by allowing to stand overnight or by placing in an oven for a short time. Place the Gooch crucible and its contents into a 50 cc. beaker and add 30 cc. of water. Boil for 10 minutes, stirring up the asbestos pad. Filter immediately through a fast filter and evaporate the filtrate to 10 cc. To 1 cc. of the filtrate in a small test tube, add 1 to 2 drops of Millon's or Stokes acid mercuric nitrate reagent or basic lead acetate solution. A precipitate having the characteristics of those listed in Table 17 confirms the presence of gums. To 3 cc. of the filtrate add 12 cc. of 95 per cent alcohol, mix and add 3 cc. of a mixture of 5 cc. of hydrochloric acid and 95 cc. of 95 per cent alcohol. Mix and add 3 cc. of water, a flocculent or stringy precipitate indicates the presence of gums. To 5 cc. of the filtrate add 5 cc. of hydrochloric acid and a few crystals of phloroglucinol and boil for 2 minutes. The production of a pink to red color, fading to amber and the subsequent formation of a brown precipitate confirms, in another manner, the presence of gums.

This method may be applied to such divers food products as cheese, mayonnaise, ice cream, smoked or canned meats and cake coatings. The concentration of the gum is again a factor and unless definite flocculent or stringy precipitates are obtained by the alcohol precipitation, gums in quantities over 0.05 per cent are not indicated. Mere turbidities are to be disregarded.

It is well to note that the confirmatory tests eliminate the interference of starch and dextrin, for these substances will not give precipitates with the Stokes acid mercuric nitrate or with the basic lead acetate reagents.

Separation of Gums

The problem of identifying gums in the presence of one another is rather difficult and, at times, impractical. It becomes important when it is necessary to establish the purity of a product during a commercial

transaction, in order to ascertain if a cheaper gum has replaced in whole or in part a more costly one.

The presence of one gum in another has been customarily established by measuring the viscosity of a solution containing a weighed amount of the sample.[16] If the viscosity is altered much, the presence of another gum is indicated. Some chemical tests have been developed as, for example, the detection of oxidase [17] as a test for the presence of arabic in tragacanth, or the estimation of the volatile acid number, calculated as acetic acid, for distinguishing between karaya and other gums. However, the acid value of gums varies so much that a large adulteration could at times be made without detection by this test alone. These methods do not tell the type of gum present.

It is obvious that very complex mixtures of gums will not ordinarily be encountered, and that if mixtures are made, they will most likely consist of two or three gums. It is possible to effect a more or less definite separation of simple mixtures by making use of the characteristic reactions of the gums. Thus, assuming the mixture to be arabic and tragacanth, precipitate the tragacanth by the addition of neutral lead acetate and filter. The filtrate contains the arabic which may subsequently be precipitated by alcohol. Dissolve the residue from the neutral lead acetate precipitation, which contains the tragacanth, in saturated ammonium acetate solution and recover the tragacanth by the addition of alcohol, which precipitates the gum alone. Filter the alcoholic precipitates; wash the precipitates on the filter paper or Gooch crucible with alcohol; dissolve the gums in hot water as described in the preceding section and then apply the tests for the characteristic reactions.

In a similar manner by precipitating locust kernel, quince seed, pectin, or Irish moss by neutral lead acetate solution and filtering, separations can be made of the following combinations: arabic and locust kernel, arabic and quince seed, arabic and pectin, arabic and Irish moss. The residue contains one of the aforementioned gums and the filtrate again contains the arabic. By dissolving the lead acetate precipitate in saturated ammonium acetate and by the subsequent addition of alcohol, the gums may be recovered.

Entirely analogous types of separation may be made in 10 combinations of quince seed gum and other gums by precipitation of the quince seed by zinc chloride or ammonium molybdate reagent and subsequent

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[16] Middleton, *Quart. J. Pharm.* 9, 506 (1936).

[17] Ritsema, *Pharm. Weekblad* 72, 105 (1935).

filtration. The filtrate contains the other gum and the residue contains the quince seed gum.

2) combinations of Irish moss or quince seed gums and other gums by precipitation of the Irish moss and quince seed with saturated barium chloride solution and subsequent filtration. The filtrate contains the other gum and the residue the Irish moss or quince seed.

3) combinations of pectin and other gums by saponification and hydrolysis of the pectin, as described in the chapter on jams and jellies, Chapter XI, with the formation of digalacturonic acid and separation by filtration. The precipitate is digalacturonic acid and the filtrate contains the other gum.

4) combinations of locust kernel and other gums by precipitation of the locust kernel with tannic acid solution and separation by filtration. The residue consists of the precipitate and the filtrate contains the other gum.

These separations are, very likely, not quantitative, because of the great similarity of the gums, both chemically and physically. It is possible in these precipitations, that some of a gum, presumably soluble, is adsorbed and will appear with the precipitate and that, on the other hand, some of the gum, presumably precipitable, remains in solution or suspension. Consequently, only after recovery of the gum by precipitation by alcohol or acetone, which frees the gum from interfering materials, by re-solution of the gum in water, and by the application of the characteristic reactions to the gum can conclusions be drawn as to the nature and type of gums composing the original mixture.

CEREAL

The proximate analysis of cereals for total solids, ash, protein, fat or ether extract, crude fiber, etc., may be made using methods described in other sections of the text. Often the percentage of carbohydrate is estimated by difference, that is, the amount of water, ash, fat and protein are determined, these percentages are added, and the sum is subtracted from 100. The remainder is assumed to be carbohydrate. A method for starch is detailed.

Grain and stock feeds may also be estimated by these methods. The methods for pentoses and galactans are detailed.

Tables 19, 20 give the proximate analysis of cereals and flour. In Table 20 the composition and comparison of various flours with that of soy flour is given. It is to be noted, as Table 21 shows that although

soy flour contains comparatively little starch, it does contain other carbohydrates. It also contains a very large amount of protein in comparison with other flours.

TABLE 19. ANALYSES OF CEREALS.[18] (UNITED STATES)

Type	Water %	Ash %	Protein %	Fat %	Crude Fiber %	Carbohydrate %
Barley						
Maximum	12.96	2.95	13.83	2.42	5.62	73.47
Minimum	8.92	1.65	8.32	1.89	1.57	66.75
Average	10.80	2.44	10.69	2.13	4.05	69.89
Buckwheat						
Maximum	13.00	2.23	11.90	2.43	12.45	64.14
Minimum	11.75	1.63	9.19	1.74	9.57	61.01
Average	12.15	1.89	10.75	2.11	10.75	62.33
Corn						
Maximum	12.32	1.55	11.55	5.06	2	75.07
Minimum	9.58	1.19	8.58	2.94	1	68.97
Average	10.98	1.71	9.88	4.17	1.71	71.95
Sweet Corn[19]	8.44	1.97	11.48	8.57	2.82	66.72
Rye						
Maximum	11.45	2.41	18.99	2.30	2.50	75.36
Minimum	9.54	1.71	8.40	1.16	1.65	63.61
Average	10.62	1.92	12.43	1.65	2.09	71.37
Wheat						
Maximum	14.53	2.35	17.15	2.50	3.72	76.05
Minimum	7.11	1.40	8.58	0.28	1.70	66.67
Average	10.62	1.82	12.23	1.77	2.36	71.18

TABLE 20. COMPOSITION AND COMPARISON OF FLOURS [20]

Flour	Water %	Protein %	Fat %	Carbohydrate %	Fiber %	Ash %
Soybean "Aguma"	8.90	49.01	8.55	26.28	1.47	5.79
Soybean "Aguman"	10.58	55.90	2.32	24.26	1.77	5.17
Soybean "Soyama"	....	42.00	18.00	24.00	....	6.00
Soybean "Berczeller"	....	45.50	22.38	....	....	4.81
Soybean Bollman	10.7	51.2	0.1	28.8	3.1	6.1
Soybean Deming	5.26	44.64	19.43	24.12	2.35	4.20
Wheat	12.00	11.0	1.00	77.35	0.20	0.45
Whole wheat	10.90	12.00	2.00	73.05	1.00	1.05
Rye	9.00	12.00	1.50	75.85	0.65	1.10
Corn	10.00	8.50	2.70	77.10	0.80	0.90

[18] Wiley, U. S. Dept. Agr., Bur. Chem., Bull. No. 45 (1895).

[19] Wiley, U. S. Dept. Agr., Bur. Chem., Bull. No. 50 (1897).

[20] Horvath, "The Soy Bean as Human Food," Peking Union Medical College (1925).

TABLE 21. COMPOSITION OF SOYBEAN FLOUR

Moisture	7.65[21]	
Protein	40.65	40.8[22]
Fat	20.38	18.3
Starch	traces	2.6
Dextrin	0.00	
Sucrose	5.26	
Reducing soluble carbohydrates	0.00	
Unusable carbohydrate	....	18.0
Stachyose	5.66	
Araban	4.83	
Galactan	6.18	
Cellulose	1.63	
Ash	6.08	
Phosphatides calculated as lecithin P_2O_5 x 11	3.08	

STARCH

To obtain satisfactory results with the following A. O. A. C. tentative method for starch in flour, the directions must be followed carefully in every detail. As the steps are timed, it is essential to learn the procedure so that no time will be lost in following the method. Arrange everything needed in the determination before the hydrochloric acid is added to the sample.

To prepare the hydrochloric acid reagent, mix approximately equal volumes of hydrochloric acid and water and adjust by titration so that 100 cc. of this solution contains 20.5–21.0 g. of hydrogen chloride.

Determination: Weigh accurately a sufficient quantity of finely ground sample, which should readily pass through a 20-mesh sieve, to represent 0.5–1.0 g. of starch. Transfer to a funnel fitted with a 9 cm. Schleicher and Schüll No. 589 or Whatman No. 40 filter paper and extract by nearly filling the filter 4 times with ethyl ether; likewise extract with alcohol, 70 per cent by volume, and with water. Allow to drain 1 hour uncovered. Transfer the drained filter and contents to a 50 cc. beaker. In the next step use a stirring rod having a flattened button-like end 15 mm. in diameter, and, which is very important, tamp with a twisting motion during the time specified in order to get the filter paper completely disintegrated and thus insure the complete suspension of the starch in the hydrochloric acid solution but not to hydrolyze any of it.

21 Kupelwieser, "Veredeltes Soyamehl. Das Oesterreichische Gesundheitswesen," p. 198 (1932).

22 Kellogg, "Soybean Flour," Battle Creek Food Co.

Complete the maceration while there is a small amount of hydrochloric acid present and the whole contents is a rather thick paste. If this optimum condition is obtained practically duplicate results will follow. Add the hydrochloric acid reagent at 15° C. to the beaker containing the sample, using a fast delivering 10 cc. Mohr pipette with 1 cc. marked off at the lower end with heavy pencil marks. Keep the acid supply on the bench, but do not allow it to get above 18° C.

Proceed as follows, adding the hydrochloric acid in the quantities given: Add 1 cc., tamp 1 minute; add 1 cc., tamp 2 minutes; add 1 cc., tamp 2 minutes; add 1 cc., tamp 1 minute; add 1 cc., tamp 1 minute; add 1 cc., tamp 1 minute; add 1 cc., tamp 1 minute.

Fill the beaker half full with the acid and stir 30 seconds. Fill the beaker ¾ full and stir 30 seconds. In the 10 minutes during this treatment the paper should be completely disintegrated and in a smooth state of suspension, the tamping should be continued vigorously during this time, and as little time as possible should be spent adding the acid. Immediately transfer to a 100 cc. wide-mouthed volumetric flask, rinsing out the beaker with the hydrochloric acid; carefully make to volume with the hydrochloric acid reagent and add 0.5 cc. for the volume of filter paper. This step should require 2 minutes. Shake the stoppered flask vigorously for 5 minutes, and allow to stand for 5 minutes in a beaker of water at 20° C. Shake twice and filter immediately into a 250 cc. suction flask through a small Büchner funnel, 41 mm. in diameter, fitted with a thin layer of asbestos and filled half full with dry, fluffy asbestos. The filtration requires 1 minute only. Immediately pipette 50 cc. of the filtrate into a tall form 200 cc. beaker containing 115 cc. of 95 per cent alcohol. The quantity of starch finally weighed will then vary from 0.25–0.5 g. The time consumed from the initial addition of the acid is 24 minutes. Allow the pipette to drain completely and then stir with a whipping motion for 1 minute to flocculate the precipitated starch. Wash down the sides of the beaker with 70 per cent alcohol. Allow to stand 3–4 minutes, until nearly all the precipitate has settled, and then carefully decant the supernatant liquid, which is somewhat turbid, so that little or no precipitate passes into the weighed Gooch crucible, which has been fitted with a thin pad of ignited asbestos and is half filled with fluffy ignited asbestos. Wash the precipitate, and filter by decantation, using successively two 40 cc. portions of 70 per cent alcohol, then 4 times, using 30 cc. portions of 95 per cent alcohol, each time breaking up the precipitate by rapid stirring and allowing the precipitate to settle before decantation. After each stirring rinse the sides of the beaker with a

small stream of alcohol to prevent the starch from drying and sticking. Finally transfer the starch completely by means of a jet of 95 per cent alcohol and wash the sides of the Gooch and precipitate with a little of the alcohol. All these filtrations are very fast. Dry the crucible and contents uncovered for 2 hours at 130° C.; cover the crucible immediately and place in a desiccator charged with phosphorus pentoxide, fresh sulfuric acid, or freshly ignited calcium oxide; cool 10 minutes and weigh. Multiply the result by 2 and report as starch.

Pentosans

The determination of pentosans, for practical purposes may be said to depend upon their conversion to furfural or to methylfurfural by distilling with a mineral acid of the proper concentration and subsequent determination of the furfural by oxidation, precipitation by various reagents or by colorimetric means. The A. O. A. C. recommends precipitation with phloroglucinol. The precipitation of furfural with phloroglucinol is not quantitative, and if hydroxymethylfurfural which is of hexose origin is present a small amount of this substance is precipitated along with the furfural. Furthermore, the phloroglucide of furfural is not a compound of definite composition, hence this method has serious errors.

Barbituric acid also precipitates furfural and is recommended by Jager and Unger [23] as better than phloroglucinol since no hydroxymethylfurfural is precipitated. Bailey [24] suggests a method based on the conversion of pentosans to furfural and methylfurfural, their separation by steam distillation and their subsequent precipitation as furfuralmalonylthiourea by means of thiobarbituric acid. Thiobarbituric acid precipitates furfural and methylfurfural quantitatively forming definite compounds of uniform composition such as furfuralmalonylthiourea and while it reacts with hydroxymethylfurfural, the resulting product is soluble, coloring the solution but not interfering with the determination of pentoses.

Determination: Place from 0.1 to 3 g. of material according to the pentosan content into a 125 cc. distillation flask and add 50 cc. of 12 per cent hydrochloric acid. Distill with steam, passing in a stream of steam at a moderate, constant rate throughout the distillation. The temperature,

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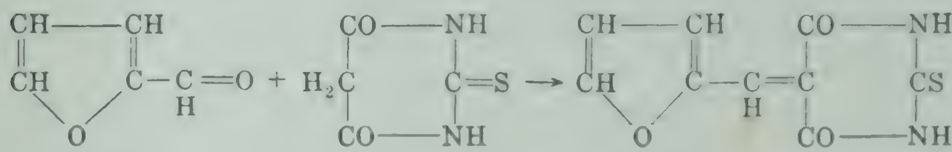
[23] Jager and Unger, *Ber.* 36, 1222 (1903).

[24] Bailey, *Ind. Eng. Chem., Anal. Ed.* 8, 389 (1936).

as measured by a thermometer in the vapor in the neck of the flask, should be maintained between 103 to 105° C. by boosting the distillation flask with a burner. Distillation is continued until a small sample of the distillate in thiobarbituric acid solution gives no precipitate or turbidity after standing 5 minutes. Aniline acetate paper is worthless for any hydroxymethylfurfural formed from hexoses present will give a positive reaction with this reagent. The furfural is precipitated from the distillate by adding a slight excess of thiobarbituric acid in 12 per cent hydrochloric acid at room temperature and allowing to stand overnight. The lemon-yellow compound formed may be filtered on a tared Gooch crucible, dried at 105° C., and weighed as furfuralmalonylthiourea.



furfural — thiobarbituric acid — furfuralmalonylthiourea

Values obtained in the presence of methylfurfural are only very slightly in error, because the molecular weight of the residue is between furfural and methylfurfural. Calculations may be based on the following relationships:

$$\text{Pentosan} \rightarrow \text{furfural} + 2H_2O$$

$$\text{Weight of pentosan} = \frac{\text{furfural} + 2H_2O}{\text{furfuralmalonylthiourea}} = 59.6\%$$

that is:

$$\text{Weight of pentosan} = 0.596 \times \text{weight of furfuralmalonylthiourea.}$$

Galactan

Galactans yield galactose on hydrolysis and galactose may be converted to mucic acid by oxidation with nitric acid under the proper conditions. These facts are the basis of Tollen's method for the estimation of galactan for which the A. O. A. C. gives the following details.

Extract a convenient quantity of the sample, representing 2.5–3 g. of the dry material, on a hardened filter with 5 successive portions of 10 cc. of ether; place the extracted residue in a beaker, about 5.5 cm. in diameter and 7 cm. deep; add 60 cc. of nitric acid (sp. gr. 1.15), and

evaporate on a steam bath to a volume of 20 cc. Let stand 24 hours, then add 10 cc. of water and allow to stand another 24 hours. Pass through a filter and wash the impure mucic acid crystals with 30 cc. of water to remove as much of the nitric acid as possible, and return the filter and contents to the original beaker. Add 30 cc. of ammonium carbonate solution (consisting of 1 part ammonium carbonate, 19 parts water, and 1 part of ammonium hydroxide) and heat the mixture in a water bath, at 80° C., for 15 minutes, with constant stirring. The ammonium carbonate combines with the mucic acid, forming soluble ammonium mucate. Wash the filter paper and contents several times with hot water by decantation, passing the washings through a filter paper, to which finally transfer the residue, and wash thoroughly. Evaporate the filtrate to dryness on a water bath, avoiding unnecessary heating which causes decomposition; add 5 cc. nitric acid (sp. gr. 1.15); stir the mixture thoroughly; allow to stand for 30 minutes. Collect the precipitated mucic acid on a weighed Gooch crucible or other filter; wash with 10–15 cc. of water, then with 60 cc. of 95 per cent alcohol, and then a number of times with ether; dry at the temperature of boiling water for 3 hours; and weigh. Multiply the weight of the mucic acid by 1.33 to convert to galactose and by 1.20 to convert to galactan.

These factors are empirical and are based on the investigational work of Tollens.[25]

REFERENCES

Wood and LaWall, Dispensatory of United States of America, 21st ed., Lippincott (1926).

Allen's Commercial Organic Analysis, 5th ed., Vol. I, Blakiston (1923).

Pharmacopoeia of the United States XI and X Decennial Revision.

Methods of A. O. A. C. (1935).

[25] Browne, "Sugar Analysis." Wiley (1912).

CHAPTER XI

JAMS, JELLIES AND FRUITS

JAMS, preserves and jellies may on the one hand be classed with sugar foods and on the other with fruits and vegetables. Their characteristic properties may be more easily determined from their relationships with fruits rather than with sugars. The methods for the analysis of syrups, maple products, honey and confectionery have received a great deal of attention and are well adapted for their purpose. On the other hand, those developed for jams, preserves and jellies although simple are time consuming and require much interpretation. This is due to the wide variation in the types and kinds of fruit and fruit combinations making the jam or jelly product.

JAMS AND PRESERVES

These products are made by cooking properly prepared fresh fruit, cold-pack fruit, canned fruit, or a mixture of two or all of these, to a suitable consistence, with sugar or with sugar and dextrose, with or without water. In its preparation not less than 45 pounds of fruit should be used to each 55 pounds of sugar or of sugar and dextrose. A product in which the fruit is whole or in relatively large pieces is customarily designated a preserve rather than a jam. In marmalades the whole fruit is pared, the pits are removed and the fruit is cooked in small pieces with sugar to a thicker consistency than jam. Orange marmalade, generally contains slices of fruit and peel, giving the product a characteristic appearance. By far the greatest proportion of jams and preserves is in solution, hence their composition differs little from that of jellies. The consistency desired in jams and preserves is that of a paste whereas that of jellies is semi-solid.

JELLY

Jellies are prepared by mixing fruit juices with sugar and evaporating to the jellying point. The formation of the jelly is due to the coagulation of pectin in a mixture of sugar, acid and water. In order to make a good jelly it must have a proper amount of sugar, water, fruit acid and pectin.

The Food and Drug Administration defines a fruit jelly as the semi-solid, gelatinous product made by concentrating to a suitable consistence the strained juice or the strained water extract from fresh fruit, from canned fruit or from a mixture of two or of all of these, with sugar or with sugar and dextrose.

The definition does not include adding pectin and according to the exclusion paragraph of Food and Drug Administration definitions, no pectin is in reality permitted to be added. Nevertheless for the production of a good jelly with some fruits it is almost essential to add a small amount of pectin. However, if pectin is added, such addition should be declared.

Forms

Jams and jellies may be adulterated in a number of ways. One is the addition of thickening agents such as added pectin, gums, gelatin, dextrins, and like products. Secondly, waste or rather exhausted material may be added. Thirdly, foreign substances may be added such as color, preservatives, saccharin, fruit acids, lactic acid, mineral salts or acids and many other substances. Fourthly, there may be a fruit shortage or conversely a sugar increase. For the detection of gums, gelatin, dextrins, color, preservatives, saccharin and mineral salts the methods previously detailed in the book may be applied. The other types of adulteration may be detected by determining insoluble solids, soluble solids by refractometer, alcohol precipitate, pectic acid, ash, and the phosphorus pentoxide content especially in grape products.

Preparation of Solution

Weigh 300 g. of the jelly or jam, both of which have been thoroughly mixed by passing the jam or preserve through a food chopper, and transfer the weighed portion with the aid of 800 cc. of water to a 2 liter beaker. Boil or heat on a steam bath for an hour or two, replacing the water lost by evaporation. Do not boil if sucrose is to be determined. Transfer the mixture to a 2 liter volumetric flask, allow to cool, dilute to volume and filter through a fluted filter.

Total Solids

Total solids may be determined in the usual manner by weighing about 20 g. of the well mixed original sample into a tared dish and drying in vacuo, at 70° C. However, for all practical purposes the refractive

index of the jam or jelly may be taken and the percentage sugar equivalent to this refractive index as given by the sugar table. Table 3 appendix, may be considered as equivalent to the total solids, with correction in some cases for the insoluble solids. Obtaining the total solids in this manner is equivalent to obtaining soluble solids.

Soluble Solids—The percentage soluble solids may also be obtained by the refractometer method and correction for insoluble solids may be made by the following formula when necessary.

$$S = \frac{a\,(100 - b)}{100}$$

where,

S = per cent soluble solids
a = per cent soluble solids as determined by refractometer.
b = per cent insoluble solids.

Insoluble Solids—Weigh 25 g. of the original well mixed sample into a 400 cc. beaker and add 200–250 cc. of water. Boil for 30 minutes and replace the water lost from time to time. Prepare a low flat-bottomed dish with a cover and a sheet of 15 cm. filter paper by drying to constant weight in an oven at 100–105° C. Filter the hot mixture through the weighed filter paper and wash thoroughly with hot water. The washing may be considered finished when no positive Molisch reaction is obtained or when the wash water no longer reacts acid to litmus paper. Replace the filter paper and its contents in the dish, replace the dish and contents in the oven and dry to constant weight. The increase in weight may be calculated as per cent insoluble solids.

This determination is not usually performed on jellies because these products are presumed to be prepared from the strained juice and therefore should contain practically no insoluble solids.

Alcohol Precipitate

This determination ascertains the amount of combustible alcohol insoluble material present in the preserve or jelly. Transfer 100 cc. of the prepared solution by means of a pipette to a tall form 300 cc. beaker and evaporate the solution to 20 cc. If any insoluble matter appears, add 1 or 2 lumps of cube sugar or 4–8 g. of granulated sugar and continue the evaporation to 20 cc. Place 200 cc. of 95 per cent alcohol in a separa-

tory funnel or similar apparatus and add the alcohol to the mixture in a thin stream, with constant stirring. Allow the mixture to stand until the flocculent precipitate settles or overnight. Filter through a smooth 15 cm. filter paper. Wash the precipitate with alcohol. Wash the precipitate back into the original beaker with a stream of hot water and wash the filter paper thoroughly with hot water catching the washings in the beaker. Again evaporate the solution to 20 cc. Cool and add 5 cc. of hydrochloric acid (1:2.5). Reprecipitate with 200 cc. of 95 per cent alcohol as directed above. Allow to stand until the flocculent precipitate settles or overnight and filter through a tared Gooch crucible which has a thin pad of asbestos, if the precipitate is small. Wash thoroughly with 95 per cent alcohol until free of hydrochloric acid. If the precipitate is large enough to clog the Gooch crucible, filter the precipitate on filter paper. Wash well with alcohol and then wash the precipitate into a tared platinum dish with a stream of hot water. Wash the filter paper well with hot water and catch the washings in the platinum dish. Evaporate the precipitate and washings in the platinum dish to dryness and then dry the dish to constant weight. If a Gooch crucible was used, dry to constant weight also. Ignite the dish or crucible and after cooling in a desiccator weigh again. The loss in weight is the alcohol precipitate.

The alcohol precipitate is sometimes almost invisible but rarely so when the jam or jelly contains dextrins or sometimes starch. Therefore care must be taken in the various transfers of the precipitate. If the precipitate does not flocculate properly, the addition of sodium chloride will aid in the flocculation. This determination should be made in duplicate.

Pectic Acid

This determination estimates the amount of digalacturonic acid or similar acids obtained from the alcohol precipitate. Transfer 200 cc. of the prepared solution to a 300 cc. tall form beaker and evaporate to 25 cc. If a precipitate forms during the evaporation, add 2–4 lumps of cube sugar or 8 to 16 g. of granulated sugar. Continue the evaporation to 25 cc. Precipitate with 200 cc. of 95 per cent alcohol as directed in the alcohol precipitate determination and allow the precipitate to flocculate and settle or to stand overnight. Filter through a 15 cm. smooth filter paper. Wash well with alcohol. Return the precipitate to the original beaker by washing with a stream of hot water. Wash the filter well with hot water and catch the washings in the beaker. Evaporate to 40 cc. Cool to room temperature. If any insoluble matter appears, add 1 cc. of 10

per cent hydrochloric acid, warm if necessary to effect solution and cool again. Add 2 to 5 cc. of 10 per cent sodium hydroxide according to the volume of the precipitate. Dilute to 50 cc. with water and allow to stand for 15 minutes. At the end of this time, add 40 cc. of water and 10 cc. of hydrochloric acid (1 : 2.5) and boil for 5 minutes. Filter on a smooth 15 cm. filter paper and wash thoroughly with hot water. Wash the precipitate back into the beaker with a stream of hot water and adjust the volume to 40 cc. either by the addition of water or by evaporation to that volume. Allow to cool and repeat the saponification and precipitation detailed above. Filter again through the same filter paper and wash thoroughly with hot water, until free of acid. The filtrations should proceed rapidly and the filtrates should be clear. Wash the filtrate into a tared platinum dish with a stream of hot water. Evaporate to dryness and then dry to constant weight in an oven at 100°–105° C. Allow to cool and weigh. Ignite and weigh again. The loss in weight is equivalent to the pectic acid. If the precipitate is not too voluminous, it may be filtered the second time through an ignited and tared Gooch crucible with a thin pad of asbestos.

This determination should be made in duplicate. If the saponification goes improperly due to insufficient alkali and colloidal precipitates are formed so that the filtration is not rapid and the filtrates are not clear the determinations should be repeated.

Total Acidity

This determination may be made in the usual way by titrating 25 cc. of the prepared solution diluted with 200 to 250 cc. of recently boiled and cooled water with 0.1 *N* sodium hydroxide solution using phenolphthalein as an indicator. If despite the high dilution the solution to be titrated is still colored deeply enough to obscure the endpoint, the titration may be made using phenolphthalein powder as an outside indicator. The result is generally calculated as cc. of 0.1 *N* sodium hydroxide solution per 100 g. of the original material.

Ash

Evaporate to dryness in a tared porcelain dish or a silica dish 100 cc. of the prepared solution. Ash the residue in the usual manner at not more than dull red heat. This determination gives total soluble ash. If the total ash is desired, 10 to 25 g. of the original well mixed sample

should be weighed into a porcelain or silica dish and then ashed as usual. Platinum dishes should not be used especially where an ash high in phosphoric acid is suspected.

Alkalinity of Ash—The alkalinity number of an ash is defined as the number of cc. of N acid required to neutralize 1 gram of ash. To the ash as determined in the foregoing section an excess of 0.1 N hydrochloric acid is added and the mixture is warmed. Cool and back titrate with 0.1 N sodium hydroxide solution using methyl orange as the indicator. Calculate the alkalinity as indicated in the definition. This determination is valueless if added mineral acid is present.

The sulfur content and the phosphorus pentoxide content may be determined as detailed in Chapter XVII on inorganic determinations.

Interpretation of Results

As in the determination of the so-called constants of the oils and fats, the estimation of insoluble solids, alcohol precipitate, pectic acid, ash and acidity serve as the constants of jams, jellies and preserves. Fruits and vegetables as other natural products have compositions that vary within certain limits and of course products made from these natural foods according to definite specifications such as preserves, will also have compositions or constants that vary within limits. In the case of jams and jellies, if these estimations are set up as ratios, the interpretation to be derived from them is far more clear. These ratios and the implications that follow are given in the subsequent section.

Ratios

$\dfrac{\text{Alcohol precipitate}}{\text{Ash}}$ A high ratio indicates the addition of commercial glucose, or gums or more important, added pectin.
A low ratio indicates the addition of mineral matter or mineral acid or the use of over-ripe or decomposed fruit.

$\dfrac{\text{Pectic acid}}{\text{Ash}}$ A high ratio indicates added pectin and gum.
A low ratio, the same as above.

$\dfrac{\text{Insoluble solids}}{\text{Ash}}$ A high ratio indicates the use of exhausted fruit, marc or pomace. A low ratio indicates the same as above or the use of fruit juice instead of fruit.

$\frac{\text{Insoluble solids}}{\text{Alcohol precipitate}}$	A high ratio indicates the use of exhausted fruit, marc or pomace. A low ratio indicates the addition of pectin, gum or commercial glucose or the use of fruit juice instead of fruit.
$\frac{\text{Insoluble solids}}{\text{Pectic acid}}$	The same as above, but not influenced by commercial glucose.
$\frac{\text{Insoluble solids}}{\text{Total acid}}$	A high ratio indicates the use of exhausted fruit, marc or pomace. A low ratio indicates added acid.
$\frac{\text{Total acid}}{\text{Ash}}$	A high ratio indicates added acid. A low ratio indicates added mineral matter.
$\frac{\text{Alcohol precipitate}}{\text{Pectic acid}}$	A high ratio indicates the addition of commercial glucose and starch, and of some gums. Other gums are hydrolyzed in a manner similar to pectin and consequently do not influence the ratio.

It is of course, obvious, that in the case of jellies the ratios involving the determination of insoluble solids are valueless, for in the methods of manufacture all of the insoluble solids are presumed to be strained out. On the other hand these ratios are very important in jams and preserves because of the indication of added exháusted fruit or marc or pomace.

In Tables 29, 30, 31, 32, 33, 34, and 35 the normal range of composition of fruits is tabulated. Since the proportion of fruit to sugar should be at least 45 lbs. to 55 lbs. and preferably more fruit to sugar, the constants of the fruit are about twice as great as the constants of the jams and preserves. However the ratios of these constants remain practically unaffected. Tables 22, 23, 24, 25 and 26 give the composition of authentic jams and jellies.

The sophistication of jams and jellies may reach such a high point through the addition of exhausted material to raise the water insoluble solids content and the addition of mineral salts to raise the ash and at the same time allow the ratio to remain normal, that reference to the ratios alone may be insufficient and the normal range of composition of the fruits themselves must be taken into consideration. Jams and jellies

containing little or no potassium indicate the lack of fruit juice. The absence of the characteristic acid of the fruit shows the absence of that fruit.

TABLE 22. ANALYSES OF AUTHENTIC JAMS [1,2]

Type	Insoluble Solids %	Soluble Solids %	Total Solids %	Alcohol Precipitate %	Total Sugars %	Sugar-free Solids %	Ash %
Apricot....	1.72	....	70.15	1.14	64.96	5.19	0.35
Currant....	6.32	....	66.32	0.92	54.09	12.23	0.84
Orange....	0.09	....	65.44	0.54	59.33	3.55	0.14
	0.89	....	72.76	1.76	68.26	4.50	0.21
	1.32	....	67.99	1.42	63.11	4.88	0.29
Peach......	1.08	....	67.33	1.49	61.86	5.47	0.28
Plum......	0.96	....	70.19	1.09	63.25	6.94	0.26
	2.57	....	64.78	1.63	57.66	7.12	0.32
(stone free)	0.79	68.36	69.15	2.6	68.4	....	....
(stones)...	7.00	46.06	53.06	1.5	44.2	....	....
Raspberry..	4.24	50.52	54.76	2.80	51.0	....	....
	5.85	63.00	68.85	1.04	58.2	....	....
Strawberry	1.90	....	69.16	0.9	61.07	8.09	0.34
	2.21	71.08	73.29	1.4	73.6	....	....

The terms, jam, preserve or jelly, unqualified imply that the fruit product conforms to federal government definitions. However, if qualified by the descriptive terminology of the following definitions no fraudulent practice can be presumed. These products are made with commercial glucose.

Glucose fruit preserve, corn syrup fruit preserve, glucose fruit jam, or corn syrup fruit jam is the product made by cooking to a suitable consistence properly prepared fresh fruit, cold-pack fruit, canned fruit, or a mixture of two or all of these, with glucose or corn syrup. In its preparation not less than 45 pounds of fruit are used to each 55 pounds of glucose or corn syrup.

Glucose fruit jelly or corn syrup fruit jelly is the semi-solid, gelatinous product made by concentrating to a suitable consistence the strained

[1] Munson, U. S. Dept. Agr., Bur. Chem., Bull. No. 66 (1902).

[2] Canada Inland Revenue Dept. Bull. No. 96 (1904).

TABLE 23. ANALYSES OF AUTHENTIC JELLIES [3,4]

Type	Total Solids	Acidity as H_2SO_4	Total Sugars	Alcohol Precipitate	Sugar-free Solids	Ash	Alkalinity as K_2CO_3
Apple......	62.67	0.24	59.53	0.81	3.11	0.10	0.15
	66.06	0.61	63.05	1.13	2.90	0.26	0.25
	60.86	0.69	55.63	1.30	5.23	0.35	0.32
	60.90	0.13	56.4	3.40	4.50	0.56	0.47
Crab apple .	61.12	0.045	59.2	0.95	1.92	0.30	0.20
Cranberry..	54.76	0.054	51.4	1.50	3.36	0.16	0.12
Currant....	64.21	1.36	59.00	3.36	5.14	0.44	0.42
	62.46	1.06	59.90	1.39	2.52	0.27	0.26
	66.22	0.92	65.70	1.18	0.52	0.22	0.22
	70.31	0.93	65.79	2.11	4.43	0.33	0.31
	75.03	1.15	69.25	2.87	5.78	0.47	0.44
	68.77	1.08	64.49	1.70	4.28	0.41	0.39
Red currant	66.78	0.098	65.7	1.25	1.08	0.44	0.32
	65.76	0.031	67.4	0.65	....	0.17	0.11
Grape......	70.66	0.31	64.13	1.25	6.53	0.21	0.28
	72.22	0.83	67.74	2.49	4.48	0.35	0.23
Guava.....	79.97	0.47	74.89	1.36	5.08	0.45	0.41
	79.14	0.47	75.91	1.59	4.23	0.38	0.35
Lemon.....	64.62	0.46	61.49	0.73	3.13	0.21	0.19
Raspberry..	70.07	0.33	63.25	1.45	6.82	0.24	0.26
	74.53	0.83	70.59	0.77	3.94	0.31	0.24
Strawberry	68.11	0.29	63.07	0.89	5.04	0.21	0.21
	67.50	0.29	63.56	1.47	5.44	0.21	0.31

[3] Munson, U. S. Dept. Agr., Bur. Chem. No. 66 (1902).
[4] Canada Inland Revenue Dept. Bull. No. 96 (1904).

TABLE 24. MAXIMA, MINIMA AND AVERAGE COMPOSITION OF AUTHENTIC JAMS AND JELLIES [5]

	Jellies			Jams		
	Average	Maximum	Minimum	Average	Maximum	Minimum
Total solids %	67.13	80.28	45.56	65.98	82.46	50.43
Insoluble solids %	....	....	....	1.92	6.32	0.09
Protein %	0.21	0.42	0.07	0.43	1.41	0.18
Acidity % H_2SO_4	0.63	1.57	0.17	0.54	1.36	0.16
Sugars						
Reducing %	37.07	65.52	3.95	36.41	61.02	13.20
Sucrose %	25.96	65.22	3.47	22.15	54.23	0.30
Alcohol precipitate %	2.53	3.36	0.73	1.12	1.76	0.09
Sugar-free solids %	4.05	6.82	0.52	7.71	14.58	3.55
Ash %	0.34	0.73	0.10	0.32	0.84	0.14
Alkalinity of ash as % K_2CO_3	0.30	0.51	0.15	0.26	0.60	0.10

TABLE 25. RANGE OF ALCOHOL PRECIPITATE CONTENT OF JELLY [6]

	Minimum %	Maximum %	Average %
Apple	0.81	1.30	1.08
Currant	1.18	3.36	2.10
Grape	1.25	2.49	1.87
Guava	1.36	1.59	1.47
Lemon	....	....	0.73
Strawberry	0.89	1.47	1.18
Raspberry	0.77	1.45	1.11

TABLE 26. RANGE OF ALCOHOL PRECIPITATE CONTENT FOR JAM [6]

	Minimum %	Maximum %	Average %
Apricot	....	....	1.14
Currant	....	....	0.92
Orange	0.54	1.76	1.24
Peach	....	....	1.49
Plum	1.09	1.63	1.36
Strawberry	....	....	0.90

[5] Munson, U. S. Dept. Agr., Bur. Chem., Bull. No. 66 (1902).

[6] Munson, U. S. Dept. of Agr., Bur. of Chem., Bull. No. 66 (1902).

juice or the strained water extract from fresh fruit, from cold-pack fruit, from canned fruit, or from a mixture of two or of all of these, with glucose or corn syrup.

The British [7] place some reliance on the non-sugar solids, that is, total solids minus sugar solids and more reliance on minimum insoluble solids as a true measure of the amount of fruit present.

Examination of Fruits and Jams by Lead Precipitation

The quantity of lead precipitate formed, when a lead acetate solution is added to a solution of a jam affords some indication of the amount of fruit in the sample. The method will not, by itself indicate the percentage of fruit in a jam with any more certainty than the other methods previously described. However it may be particularly useful in the analysis of jams made from a mixture of fruits, the acids of which differ, for example, one fruit containing citric acid and the other malic acid. It is sometimes useful in indicating whether a fruit juice or a commercial pectin has been used in the jam. By use of the method it is possible to place the various fruits in three classes, i. e. those containing mainly (1) citric acid, (2) malic acid, (3) lactic acid, or an acid having similar lead precipitating properties.

Hinton [8] found that the theoretical amount of 2 per cent lead acetate solution, equivalent to each 0.1 g. of acid, as citric was 13.55 cc. He found that malic acid was not precipitated by lead acetate solution but that in the presence of citric acid, lead acetate solution precipitated all of the citric acid and 43 per cent of the malic acid. Tartaric acid is also completely precipitated by lead acetate solution but lactic acid is not precipitated and, if present, would make the apparent malic acid content high. However, the lead salts of citric, malic and tartaric acids are only very slightly soluble in 50 per cent acetone, whereas that of lactic acid is soluble, consequently the amount of lactic acid may be estimated by calculation.

Determination of Lead Number—*Preparation of Extract.* Weigh 250 g. of the sample into a beaker, and add 250 cc. of water. Mix well to break up the jam, then heat to boiling with continual stirring, and boil gently for an hour, with occasional stirring, keeping the beaker covered.

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[7] Hughes and Maunsell, *Analyst* 59, 231 (1934).

[8] Hinton, *Analyst* 59, 248 (1934).

and maintaining the volume by adding water if necessary. Cool, transfer to a 500 cc. volumetric flask, make up to volume, shake well and filter through a coarse filter.

Preparation of Pectin-free Filtrate—Transfer 250 cc. of the aqueous extract to a 500 cc. flask, and add acetone, while swirling round without incorporating too much air, to the mark. Mix well, and filter through a large dry filter, with precautions to avoid loss of acetone by evaporation.

Titration of Free Acid—Pipette 40 cc. of the pectin-free filtrate into a large beaker, add about 500 cc. of boiled and cooled water, and titrate with 0.1 *N* sodium hydroxide, using phenolphthalein as indicator. Carry out a blank titration of 500 cc. of water similarly, and deduct this from the jam titration. The difference, multiplied by 0.07, gives the percentage of free acid in the jam, expressed as hydrated citric acid.

Ash of Jam—Evaporate 50 to 100 cc. of the pectin-free filtrate to dryness in a platinum dish on a water bath, char and ash at a dull red heat, preferably in a muffle. Cool and if desired weigh.

Titration of Combined Fruit Acid—Dissolve the ash in a measured 15 cc. portion of 0.1 *N* hydrochloric acid, filter into a 175 cc. conical flask, and wash through thoroughly. Boil for a few minutes. Cool, add a drop of methyl orange solution, titrate to yellow with 0.1 *N* sodium hydroxide solution, and then to the neutral tint with 0.1 *N* hydrochloric acid. Calculate the amount of acid consumed by the ash to the number of cc. of 0.1 *N* acid per 100 g. of jam. This is the methyl orange alkalinity of the ash.[9] Next acidify the titrated solution with about 2 cc. of 0.1 *N* hydrochloric acid, and evaporate to about 15 cc. It is advisable to do so on a sand bath to minimize bumping. Cool, and neutralize carefully to methyl orange with 0.1 *N* sodium hydroxide solution. Add a few drops of phenolphthalein solution and 10 cc. of a strong neutral calcium chloride solution. Boil again for a few minutes, and titrate to the phenolphthalein end point with 0.1 *N* soduim hydroxide solution. Calculate the number of cc. of 0.1 *N* sodium hydroxide solution per 100 g. of jam, and multiply by 3/2. This gives the phosphoric acid in the ash as its equivalent of 0.1 *N* sodium hydroxide solution. Then find the "total alkalinity" of the ash,

[9] Pfyl, *Z. Nahr. Genussm.* 43, 313 (1922).

equivalent to all the alkali and alkaline earth metals present, by adding to the "methyl orange alkalinity" 1/3 of the phosphoric acid equivalent. Finally multiply this total alkalinity by 0.007 to obtain its value as percentage of combined fruit acid as citric acid.

Total Fruit Acid—The total fruit acid, including any phosphates present in the extract, is the sum of the free acid and combined acid found as above, and is expressed as the percentage of total citric acid, hydrated.

Aqueous Lead Number—Take an amount of pectin-free filtrate, to the nearest 5 to 10 cc. containing approximate amounts of total fruit acids according to the following scheme:

1) Gooseberry, apricot, or blackberry jams, 0.50 g.
2) Strawberry, raspberry, red currant, or black currant jams, 0.35 g.
3) Apple, cherry, plum, greengage, or damson jams, 0.65 g.

Remove the acetone by distillation, transfer the residue to a 250 cc. volumetric flask and cool. Then add, in the case of groups (1) and (2), 3.0 cc. of 10 per cent malic acid solution, or in the case of group (3), 3.0 cc. of 5 per cent citric acid solution, pipetted accurately. The strength of the acid used should be correct to within 1 per cent of the total. Dilute to about 200 cc. Make sure that the temperature of the solution is at about 16 to 20° C., then add from a pipette, while rotating the flask, 20 cc. of lead acetate solution, containing 100 g. of normal lead acetate crystals, and 12.5 g. of acetic acid per liter, and make up to volume with water. Shake well and filter without delaying more than a few minutes. Titrate 50 cc. of the filtrate, diluted with 50 cc. of water, at or near the boiling point, with ammonium molybdate solution, 9.3 g. of ammonium molybdate per liter, using a 0.5 per cent solution of tannic acid as an outside indicator by spotting on a tile. The first appearance of a distinct yellow color in the test drop, or a definite increase in a slight existing yellowish color marks the end point.

For a blank titration, dilute 20 cc. of the lead acetate solution to 250 cc. and titrate 50 cc. of this plus 50 cc. of water in the same way. Correct the difference between the two titrations for any lack of correct strength in the lead or molybdate solutions. If the difference is not in the range 11 to 14 cc. repeat the determination on a larger or smaller quantity, as the case may require.

From the corrected titration difference deduct 3.6 cc., when malic acid was initially added, or 4.1 cc. when citric acid was added. Calculate the remainder back to the number of cc. of 2 per cent lead acetate solution, which would be completely precipitated by 10 g. of the original sample, or 40 cc. of the pectin-free filtrate. This is the lead number, aqueous, of the jam. That is, if the amount of pectin-free filtrate taken for the test be P cc. and the corrected titration difference D, then,

$$\text{Aqueous Lead Number} = L = \frac{200D}{P}$$

The "lead number per 0.1 g. of acid" is then found by simply dividing L by the percentage of total fruit acid (a) in the sample

$$l = \frac{L}{a}$$

Acetone (50 Per Cent) Lead Number—Take an amount of pectin-free filtrate, to the nearest 5 to 10 cc., containing approximate amounts of total fruit acid as follows:

1) Gooseberry, strawberry, raspberry, red currant, black currant, apricot, or blackberry jams, 0.50 g.
2) Apple, cherry, plum, greengage, or damson jams, 0.40 g.

If more than 200 cc. would be required, the amount must be restricted to this figure.

Place the required amount in a 250 cc. volumetric flask, and in case of jams of group (2) add 3.0 cc. of 5 per cent citric acid solution, measured accurately. Should it have been necessary to limit the amount of pectin-free filtrate taken to 200 cc., the deficiency of fruit acid may be made up by a suitable addition of 5 per cent citric acid solution, its effect being allowed for later.

Add acetone, while rotating the flask, to make up the total amount of acetone present to 125 cc. Then add from a pipette, 20 cc. of 10 per cent lead acetate solution, as in the aqueous test, and make up to volume with water. Mix and filter, taking precautions to avoid loss of acetone by evaporation. Titrate 50 cc. of the filtrate, diluted with 50 cc. of water, as in the aqueous test. Correct the difference between the titration and the blank for any factors of the lead of molybdate solutions. If the

difference so corrected is not approximately 14–15 cc. make a further correction as follows:

Lead titration difference cc.	Correction cc.
9.0–11	−0.3
11.1–13	−0.2
13.1–14	−0.1
14.1–15	0
15.1–15.7	+0.1
15.8–16.2	+0.2
16.3–16.5	+0.3
16:6–16.8	+0.4
16.9–17.1	+0.5

From the corrected titration difference deduct 1.5 cc. for each 1 cc. of added 5 per cent citric acid solution, if any.

Calculate the remainder, as before, back to the number of cc. of 2 per cent lead acetate solution completely precipitated by 10 g. of the sample. This is the lead number, acetone, L^1 of the jam. The lead number per 0.1 g. of the acid is then obtained as before.

$$l^1 = \frac{L^1}{a}$$

Interpretation of Results—If l^1 is appreciably less than 15, and plums or greengages, are not present, lactic acid from a commercial pectin is probably present, and its amount can be approximately found from

$$k = a - \frac{L^1}{15}$$

where k is the percentage of lactic acid in the sample. A corrected value for l is then obtained by deducting the lactic acid from the total acid.

$$l \text{ (corrected)} = \frac{L}{a - k}$$

The value for l, corrected or not as required, and the total acidity, a, with any lactic acid deducted, are then used in some of the following formulae to find the fruit content of the sample.

The lead number is due to the acid constituents of the fruits. Furthermore the acid constituents themselves, can, by means of the lead precipitation be separated into groups containing a preponderance of citric or malic acid. Thus it is possible, within certain rough limits, to discover whether the acid constituents of a jam are normal to the class of fruit used, provided that any foreign fruit or fruit juice added belongs to a different class. In particular, the addition of apple pulp or juice, or pomace extract, to strawberry, raspberry, etc., jam, should make itself evident by disturbing the normal citric acid preponderance of these fruits.

For this purpose it is not necessary to calculate from the lead number the actual quantities of citric and malic acids present. The lead numbers themselves can be used and compared with established data for the several kinds of fruits concerned, with due allowance for the natural variations. It may be pointed out, what is evident from Tables 27 and 28, that it is not the lead number itself that is specially characteristic of a particular fruit. Thus, a lead number of 6.5 found for a jam might be given by 40 per cent of strawberries alone, or by 25 per cent of strawberries and 26 per cent of apple pulp, or by 60 per cent of apple alone. The characteristic property which makes it possible to gain some idea of the proportions of the components in such mixtures is the lead number relative to the acid content, or, according to the empirical method for expressing this property, the "lead number per 0.1 g. of acid." It should be remembered that total acid is always meant here, as obtained from the free acidity and ash titrations. Hence, from a consideration of the average values for this figure for the fruits concerned, their respective proportions in an unknown mixture, or rather, the proportions of their acids, can be approximately determined.

This can be made clear by the following example. Suppose the lead number of a strawberry and apple jam be 6.4, and the percentage of total acid in the jam be 0.61, then the acid in the 10 g. of jam equivalent to the 6.4 cc. of 2 per cent lead acetate solution is 0.061 g. and the "lead number per 0.1 g. of acid" is $6.4/0.61 = 10.5$. The averages for strawberries and apples are, respectively, 12.4 and 7.1, Table 27. Hence the proportion of the acids due to strawberries is $\frac{10.5 - 7.1}{12.4 - 7.1} \times 100$ per cent of the total = 64 per cent. Thus, the percentage of acid in the jam due to strawberries, is $0.61 \times 64/100 = 0.39$ per cent; and that due to apples will, therefore, be $0.61 - 0.39 = 0.22$ per cent. Taking the average total acid

contents of strawberries and apples as 1.31 per cent and 1.33 per cent respectively, Table 27, the amounts of the two fruits in the jam are:

$$\text{Strawberry: } \frac{0.39}{1.31} \times 100 = 30 \text{ per cent}$$

$$\text{Apple: } \frac{0.22}{1.33} \times 100 = 17 \text{ per cent}$$

The same method can be applied to other mixtures, provided the characteristic figures for the fruits concerned, "lead number per 0.1 g. of acid" are sufficiently far apart. Thus the method breaks down for such mixtures as raspberry and red currant, plum and apple, etc., and is of doubtful value for blackberry and apple, for strawberry and gooseberry, as Table 27 indicates.

The calculation can be expressed in the form of a simple formula.

Let F_1 and F_2 be the respective percentages of two fruits in a mixed product,

L_1 and L_2 be the average values of the "lead number per 0.1 g. acid," Table 27,

A_1 and A_2 be the average values for total acid in the two fruits, Table 27,

l be the actual "lead number per 0.1 g. of acid" found in the sample and

a be the actual per cent of total acidity found,

Then

$$F_1 = \frac{100a(l - L_2)}{A_1(L_1 - L_2)} \qquad \text{(Q)}$$

and

$$F_2 = \frac{100a(L_1 - l)}{A_2(L_1 - L_2)}$$

Many jams on the market contain added pectin, which is used either in the form of a direct extract from pomace, or a specially prepared proprietary "fruit pectin," usually prepared from apple residues. The latter preparations, which are more or less pure pectin, should introduce no lead precipitating acids but themselves into the jam and, as they are removed along with the natural fruit pectins by the preliminary precipitation with acetone, they should cause no complications.

The case is different with pomace extracts, etc. These, when they are aqueous extracts, may be considered as apple extracts deprived of a

portion of their natural acids. The remaining acids, however, will still have the same lead-precipitating properties as the acids of the whole fruit, apart from the pectinous constituents, which can be removed before the lead test is made. Thus the formulae immediately above can still be applied, though the apparent percentage of apple juice indicated will be low because of the removal of part of its acid. There will be no interference with the calculation of the amount of the main fruit constituent, F_1.

Some commercial pectins, however, appear to have been prepared by an extraction of pomace with lactic acid. The calculation of the fruit-content from the aqueous lead number by formula (Q) would be erroneous in jams containing such pectin preparations. The effect of the lactic acid would be, as previously noted, to depress the "lead number per 0.1 g. of acid" making the proportion of apple acids appear too high. The solution of the difficulty is afforded by the second, or acetone lead number. This gives a value for the total acids excluding the lactic acid, so that a corrected value can be obtained from the "lead number per 0.1 g. of acid" which refers only to the malic and citric types of acid. Formula (Q) can then be applied to these corrected values for a and l.

This calculation is of use only for those fruits which themselves show no appreciable amount of the lactic type of acid, namely, the soft fruits and damsons. But as plums and greengages have an aqueous lead number so similar to that of apples as to preclude its use in calculating fruit-content in their case, the restriction is not a material one.

It is desirable to note in connection with the calculation of fruit-content in mixtures containing commercial pectins, experience in the analysis of a large number of jam samples of various origin has shown the figure of 7.1 for "lead number per 0.1 g. of acid" of apples, Table 27, to be rather high for general application. A figure giving results more in accordance with other analytical indications is 6.5. This too, is about the figure given by commercial pectins when due allowance is made for the extraneous lactic acid.

Hence, for the fruit-content of jams with added pectin, from apples, formula (Q) may be simplified to

$$F_1 = \frac{100a(l - 6.5)}{A_1(L_1 - 6.5)}$$

The a and l of this formula should be suitably corrected for any lactic acid shown to be present by the acetone lead number. Values of A_1 and L_1 appropriate to the various fruits may be obtained from Table 27.

TABLE 27. LEAD NUMBERS OF JAM FRUITS (AQUEOUS PRECIPITATION [10])

Fruit	Lead number cc.		Total acidity as citric acid %	Lead number per 0.1 g. acid cc.
Gooseberries (17)	max.	29.9		11.7
	min.	17.3		9.5
	ave.	25.5	2.44	10.4
Strawberries (15)	max.	22.7		13.3
	min.	9.8		10.5
	ave.	16.3	1.31	12.4
Raspberries (11)	max.	34.2		14.3
	min.	19.0		12.1
	ave.	26.8	2.02	13.3
Red currants (7)	max.	43.1		14.3
	min.	34.8		12.0
	ave.	37.6	2.81	13.4
Black currants (10)	max.	64.8		14.4
	min.	39.5		12.2
	ave.	52.7	3.92	13.5
Apples (4)	max.	10.9		7.4
	min.	6.9		6.3
	ave.	9.4	1.33	7.1
Plums (5)	max.	18.0		7.6
	min.	6.9		2.5
	ave.	10.2	2.14	4.8
Greengages (2)	1	12.0		5.9
	2	8.1		5.5
	ave.	10.1	1.77	5.7
Damsons (4)	max.	19.4		7.0
	min.	9.4		3.0
	ave.	13.0	2.77	4.7
Blackberries (12)	max.	22.5		10.2
	min.	7.2		8.1
	ave.	13.9	1.51	9.2
Apricots (2)	1	20.8		11.0
	2	14.7		9.6
	ave.	17.8	1.72	10.3

[10] Hinton, *Analyst* 59, 248 (1934).

TABLE 28. LEAD NUMBERS OF JAM FRUITS (50 PER CENT ACETONE PRECIPITATION [11])

Fruit	Lead number cc.		Total acidity as citric acid %	Lead number per 0.1 g. acid cc.
Gooseberries (2)............	1	40.9		15.4
	2	39.8		15.4
	ave.	40.4	2.62	15.4
Strawberries (3)............	max.	27.0		15.6
	min.	15.8		15.3
	ave.	21.4	1.39	15.4
Raspberries (3).............	max.	37.4		15.6
	min.	35.0		15.0
	ave.	36.6	2.38	15.4
Red currants (2)............	1	51.8		17.1
	2	50.7		16.7
	ave.	51.3	3.04	16.9
Black currants (2)..........	1	78.2		17.3
	2	74.1		16.3
	ave.	76.2	4.53	16.8
Apples (4).................	max.	22.2		15.1
	min.	16.5		14.9
	ave.	19.9	1.33	15.0
Plums (3)..................	max.	34.4		14.6
	min.	19.0		12.3
	ave.	26.1	1.88	13.9
Greengages (3).............	max.	28.6		13.0
	min.	15.0		10.3
	ave.	20.3	1.68	12.1
Damsons (2)................	1	43.3		15.6
	2	34.9		14.6
	ave.	39.1	2.59	15.1
Blackberries (2)...........	1	34.4		15.6
	2	22.2		15.2
	ave.	28.3	1.84	15.4
Apricots (2)...............	1	25.9		15.3
	2	23.5		13.6
	ave.	24.7	1.72	14.4

[11] Hinton, *Analyst* 59, 248 (1934).

FRUITS

Fruits were very likely the first vegetable food of man. Later tubers and cereals were cultivated. The water content of fruits varies from 60 to 90 per cent. The pulpy nature of the fruit protects the seeds until the spring when conditions are favorable for growth.

Fruits undergo a series of progressive changes from the unripe stage, to the ripe stage, to the rotten and fermentative stages. The ripening point of a fruit is considered to be that point at which the sugar content is a maximum. In oranges and grapefruit where many means are used to make the fruit appear ripe, maturity is based on a minimum invert sugar-citric acid ratio of eight to one. Apples are considered ripe when the last trace of starch disappears.

The browning of fruit flesh on exposure to air is due to the oxidation of the tannin in the fruit by an oxidase in the presence of the oxygen of the air. In drying fruits this effect is countered by sulfuring, that is drying by exposing to the fumes of burning sulfur. By the use of this process fruits are obtained that are not really dry and which will subsequently lose or take on moisture according to whether the fruit is stored under dry or humid circumstances.

Fruit juice is the product obtained by pressure from fruit. Fruit juices are sold as such and those more commonly encountered are apple juice or sweet cider, grape juice, orange juice, pineapple juice, grapefruit juice and tomato juice. Some of these are frequently concentrated and sold as syrups for fountain use in sodas. When allowed to ferment, these juices form the well known products, hard cider from apple juice, wine from grape juice and perry from pear cider.

Only occasionally does the food analyst analyze fruits, as such. His interest lies in the analysis of fruit products. However, it is necessary to perform many investigational determinations in order to establish more definitely the normal constants of fruits. The determinations may be made in a manner entirely similar to those previously detailed for jams and jellies. Added color in oranges and similar citrus fruits may be detected by the method detailed in coloring matters in foods, Chapter III. The vitamin C content of citrus fruits may be estimated by one of the methods outlined in the chapter on vitamins, Chapter XVI. Inorganic constituents may be estimated on the ash of the fruit by methods detailed in Chapter XVII. Spray residue determinations have been fully detailed in the chapter on metals in foods, Chapter V. Estimations of characteristic acids may be made by the subsequent methods.

Table 29. Analyses of Fresh Fruits[12]

Fruit		Total solids %	Total Sugars % as invert sugar	Pectin %	Insoluble solids %
Cooking apples	max.	15.61	8.72	1.60	2.47
	min.	10.25	3.64	0.84	1.95
	av.	13.04	7.11	1.29	2.17
Eating apples	max.	17.98	12.58	0.93	1.91
	min.	12.29	3.16	0.71	1.51
	av.	15.12	9.72	0.82	1.70
Cherries (without stones)	max.	24.70	15.30	0.54	3.10
	min.	14.74	8.28	0.24	1.29
	av.	18.64	11.47	0.35	2.05
Apricots (without stones)	max.	14.30	7.61	1.32	2.49
	min.	10.13	1.57	0.71	1.57
	av.	12.97	5.19	1.03	2.00
Blackberries	max.	18.67	4.36	1.19	10.00
	min.	13.62	2.59	0.68	6.45
	av.	16.24	3.48	0.94	8.13
Black currants	max.	24.43	7.44	1.79	6.18
	min.	15.93	3.66	1.37	4.78
	av.	19.44	5.50	1.52	5.51
Gooseberries	max.	13.90	6.54	1.20	2.76
	min.	7.93	2.00	0.95	1.66
	av.	11.38	3.98	1.08	2.26
Greengages (without stones)	max.	18.27	9.77	1.32	1.99
	min.	11.01	4.68	0.95	1.40
	av.	14.10	6.45	1.14	1.56
Plums (without stones)	max.	15.18	8.76	1.48	1.75
	min.	9.65	2.28	0.75	1.00
	av.	12.87	6.31	0.96	1.22
Raspberries	max.	24.82	8.67	0.86	6.22
	min.	12.38	2.54	0.58	4.23
	av.	16.78	4.80	0.71	5.50
Red currants	max.	20.72	7.88	1.50	5.65
	min.	12.70	2.95	0.91	3.99
	av.	16.12	5.38	1.16	4.77
Strawberries	max.	13.04	7.07	0.73	2.13
	min.	8.95	3.37	0.60	1.70
	av.	10.80	5.56	0.68	1.90
Loganberries	max.	17.11	5.92	0.68	7.25
	min.	16.69	2.66	0.62	7.13
	av.	16.92	4.04	0.65	7.19

12 Lampitt and Hughes, *Analyst* 53, 32 (1928).

Table 30. Analyses of Fresh Fruits[13]

Fruit		Total solids %	Total sugar as invert %	Non-sugar Solids %	Insoluble Solids %	Acidity No. cc. 0.1 N per 100 g.	Crude calcium pectate %	Refrac. read. of juice sugar scale %
Gooseberries	max.	14.0	7.7	9.0	2.8	415	1.2	10.1
	min.	7.9(51)	2.0(51)	4.4(51)	1.7(7)	176(36)	0.3(9)	5.2(9)
	ave.	11.2	4.4	6.8	2.3	235	0.8	7.0
Strawberries..	max.	13.2	8.2	7.4	2.4	200	0.7	10.2
	min.	8.2(145)	3.4(145)	2.9(145)	1.5(13)	90(125)	0.2(16)	6.1(74)
	ave.	10.2	5.4	4.8	1.9	145	0.5	6.6
Raspberries...	max.	21.3	8.7	17.7	6.2	390	0.9	12.3
	min.	11.0(107)	3.2(107)	7.4(107)	4.2(13)	106(90)	0.6(13)	5.3(57)
	ave.	14.4	4.8	9.7	5.4	203	0.7	7.9
Red currants .	max.	20.7	7.9	17.2	7.8	495	1.5	....
	min.	12.7(26)	2.2(26)	7.4(26)	4.0(5)	275(13)	0.9(5)	....
	ave.	16.0	5.0	10.9	5.5	375	1.1	....
Black currants	max.	24.4	10.2	17.7	6.3	622	1.8	14.5
	min.	13.7(37)	1.6(37)	10.8(37)	4.8(5)	121(23)	1.4(5)	8.0(5)
	ave.	19.0	5.0	14.1	5.7	449	1.6	11.0
Cherries...... (stone-free)	max.	24.7	15.3	9.8	3.1	145	0.5	18.3
	min.	10.9(41)	6.4(41)	3.3(41)	1.3(7)	96(27)	0.2(5)	10.0(10)
	ave.	16.3	10.2	6.1	1.9	107	0.3	13.9
Plums various (stone-free)	max.	21.9	13.3	11.7	2.0	386	1.5	22.4
	min.	8.1(91)	2.3(91)	3.4(91)	1.0(14)	25(70)	0.7(13)	10.0(36)
	ave.	14.0	7.8	6.2	1.4	215	1.2	14.1
Greengages (stone-free)	max.	21.5	13.9	11.8	2.0	435	1.4	19.7
	min.	11.0(49)	4.1(49)	5.1(49)	1.4(9)	88(39)	1.0(7)	10.2(14)
	ave.	15.6	7.9	7.8	1.5	189	1.2	16.2
Blackberries..	max.	21.2	10.4	16.0	10.5	206	1.2	11.4
	min.	14.1(29)	1.7(29)	8.4(29)	6.3(9)	90(18)	0.6(9)	6.5(12)
	ave.	16.8	4.0	12.8	8.4	135	0.8	8.5
Apricots...... (stone-free)	max.	18.4	11.8	10.4	2.5	349	1.3	18.5
	min.	8.6(55)	3.0(55)	4.1(55)	1.2(10)	123(43)	0.7(11)	8.0(24)
	ave.	12.4	5.6	6.8	1.7	235	1.0	12.9
Loganberries..	max.	23.3	7.3	22.2	7.3	420	0.7	....
	min.	13.2(19)	1.1(19)	7.3(19)	7.1(2)	151(10)	0.6(2)	....
	ave.	16.6	4.5	12.1	....	315	....	....
Apples, whole.	max.	19.5	13.5	9.8	3.4	410	1.6	17.0
	min.	10.3(147)	3.2(147)	1.1(147)	1.4(12)	25(115)	0.5(16)	9.8(39)
	ave.	15.1	10.3	4.9	2.2	162	0.8	13.4
Apples, edible portion	max.	19.0	14.2	6.9	2.3	450	1.0	17.3
	min.	11.5(80)	6.2(80)	1.4(80)	1.5(5)	20(75)	0.4(9)	9.8(38)
	ave.	15.2	10.8	4.4	2.0	93	0.6	13.5
Pears, whole..	max.	21.9	12.6	9.9	....	47	....	18.6
	min.	14.6(22)	7.3(22)	5.6(22)	....	10(23)	....	12.0(18)
	ave.	17.9	10.3	7.6		23		16.0
Pears, edible	max.	20.2	12.8	9.7	1.8	42	0.7	19.2
	min.	13.5(23)	7.8(23)	5.2(23)	1.7(3)	13(25)	0.3(3)	12.0(18)
	ave.	17.1	10.5	6.6	1.8	26	0.6	13.2

Number of samples in brackets.

These results are very similar to those of Macara.[14]

[13] Hughes and Maunsell, *Analyst* **59**, 231 (1934).

[14] Macara, *Analyst* **56**, 39 (1931).

Table 31. Analyses of Fresh Fruits[15]

Fruit	Total solids %	Protein %	Ash %	Sugars % as invert	Acid % as malic (M) citric (C)
Avocado[16]	max. 39.5	4.4	1.93	1.6	....
	min. 15.7	0.8	0.54	0.3	....
	ave. 25.9(129)[17]	2.0(112)	1.28(80)	0.7(23)	....
Banana	max. 34.6	2.0	1.4	25.7	0.55 M
	min. 16.6	0.8	0.5	14.5	0.26
	ave. 25.2(69)	1.2(59)	0.84(62)	19.2(36)	0.39(21)
Figs	max. 45.0	2.4	1.05	20.5	0.38 C
	min. 12.0	0.8	0.26	3.5	0.02
	ave. 22.0(53)	1.4(59)	0.64(68)	16.2(68)	0.17(44)
Grapefruit	max. 14.0	0.6	0.54	8.5	1.58 C
	min. 6.9	0.3	0.30	4.6	0.9
	ave. 11.2(61)	0.5(10)	0.42(8)	6.5(47)	1.17(47)
Grapes	max. 28.0	2.2	0.6	14.4	1.67 M
	min. 14.1	0.7	0.3	7.0	0.86
	ave. 18.1(28)	1.4(10)	0.45(10)	11.5(30)	1.21(14)
Guavas	max. 24.2	1.5	1.00	10.0	0.88 C
	min. 15.4	0.3	0.46	3.0	0.34
	ave. 19.4(17)	1.0(13)	0.70(17)	6.1(12)	0.62(10)
Lemons	max. 11.9	1.1	0.71	....	....
	min. 9.5	0.6	0.5	....	....
	ave. 10.7(6)	0.9(5)	0.54(6)	2.2	5.07 C
Limes	max. 14.6	0.9	1.0	0.6	7.2 C
	min. 12.4	0.6	0.7	0.3	4.2
	ave. 14.0(3)	0.8(2)	0.8(3)	0.5(3)	5.9(3)
Muskmelons	max. 12.5	1.2	1.02	11.3	....
	min. 3.5	0.2	0.2	2.4	....
	ave. 7.2(70)	0.6(11)	0.57(45)	5.4(60)	....
Peaches	max. 18.1	1.0	0.63	13.1	1.5 M
	min. 10.0	0.2	0.32	5.76	0.35
	ave. 13.1(154)	0.5(31)	0.47(31)	8.8(157)	0.64(165)
Pineapples	max. 18.9	0.6	0.7	15.3	1.10 C
	min. 9.9	0.2	0.3	8.2	0.39
	ave. 14.7(131)	0.4(46)	0.42(46)	11.9(34)	0.72(30)

[15] Chatfield and McLaughlin, U. S. Dept. Agr., Circ. No. 50 (1928).

[16] The fat content of fruits is small and varies from 0.0 to 1.5% except for avocados, which have max. 28.8, min. 7.1, and av. 17.2%.

[17] Number of samples in brackets.

Table 32. Range of Alcohol Precipitate Content of Fruits[18]

	Minimum	Maximum	Average
Strawberry	0.48	0.56	0.54
Blackberry	0.61	0.74	0.68
Cherry	....	....	0.67
Currant	....	....	0.80
Red raspberry	0.70	0.78	0.74

Table 33. Range of Alcohol Precipitate Content of Fruit Juices[18]

	Minimum	Maximum	Average
Black raspberry	1.00	1.62	1.36
Red raspberry	0.65	0.73	0.69
Strawberry	0.48	0.69	0.56

Citric Acid

This method, in contradistinction to oxidation to acetone and pentabromoacetone, consists in precipitating citric acid with barium acetate in dilute alcoholic solution to separate it from interfering substances. The precipitated citrate, which is soluble in acid is then oxidized by permanganate in the presence of mercuric sulfate, yielding a very insoluble precipitate which may be determined gravimetrically. It was developed by Bruce.[19]

Reagents and apparatus: a) A fresh solution of analyzed anhydrous citric acid containing 2 mg. of citric acid per cc.

b) A solution of mercuric sulfate, made by dissolving a suspension of 50 g. of mercuric oxide in 500 cc. of water by the gradual addition of 200 cc. of 96 per cent sulfuric acid and diluting to 1 liter.

[18] Munson, U. S. Dept. of Agr., Bur. of Chem., Bull. No. 66 (1902).

[19] Bruce. *Ind. Eng. Chem., Anal. Ed.* **6**, 283 (1934).

Table 34. Maxima, Minima and Average of Constants of Florida Oranges

	Ave.	Max.	Min.	Compos-ite	Grand Ave.
Wt. of Orange in g.	204	184	229	....	....
Wt. of Peel	54	64	41	....	....
Per cent of Peel	26.5	31.8	20.6	....	....
Wt. of Juice	86	99	64	....	....
Per cent of Juice	41.1	48.7	31.3	....	....
Wt. of Pulp	65	93	51	....	....
Per cent of Pulp	32.5	44.7	25.4	....	....
Specific Gravity @ 20/20	1.048	1.050	1.048	1.047	1.047
Per cent solids by sp. gr.	11.5	12.4	10.7	11.7	11.6
Refractive Index @ 20°C.	1.3500	1.3519	1.3484	1.3503	1.3500
Per cent Solids by Refractive Index	11.5	12.6	10.4	11.6	11.5
Per cent Solids Gravimetrically	11.94	12.6	10.6	....	....
Average Per cent Solids	11.3	12.4	10.4	11.6	11.4
Per cent Ash	.43	.45	.41	.42	.42
Alkalinity of Ash cc. N acid per g. ash	10.4	11.0	9.4	10.4	10.4
Total Acidity cc. N/10 alk. per 100 cc.	104	122	81	111.5	107.7
Citric Acid g. per 100 cc.	.67	.78	.52	.71	.69
Ratio Solids to Acid	17.7	22.9	15.0	16.3	17.0
Per cent Nitrogen	.12	.13	.12	.12	.12
Per cent Protein	.75	.80	.72	.74	.74
Mg. Vit. C per cc. of Juice	.544	.625	.440	.549	.546

Citric acid

Table 35. Analyses of Strawberries [20]

	Maximum	Minimum	Average
Ash %	0.62	0.53	0.55
Alkalinity of ash, cc. N acid per 1 g. of ash	14.2	9.6	13.0
Water Insoluble Solids %	4.10	3.00	3.46
Alcohol Precipitate %	1.07	0.70	0.82
Pectic Acid %	0.69	0.37	0.52

c) Heavy walled Pyrex glass centrifuge tubes of 15 cc. capacity, numbered, weighed, and marked at 10 cc. A copper stand holds the centrifuge tubes in a water bath and a special stirrer is used when the

[20] Wichmann, *J. Assoc. Official Agr. Chem.* 8, 123 (1924).

permanganate is added to each in turn. The stirrer is made from a 25 cm. length of 3 mm. glass rod, fitted with a glass bearing and rubber stopper as holder. The upper end is bent to a small hook and weighted. The lower end is bent to a short spiral small enough to enter the centrifuge tubes, with a 2 cm. tip to prevent the stirrer from sticking in the cone. This stirrer when mounted is operated by a string in the hand of the analyst. Graduated 1 cc. pipettes are used to measure the mercuric sulfate and phosphoric acid solutions. Potassium permanganate is added from a 10 cc. burette having a rubber connection with a glass pearl and a bent tip. This type of burette permits more accurate control of the rate of addition of the reagent than the type with a glass stop cock. A silver or platinium wire is used for stirring the precipitate in the wash liquid.

Procedure: For approximately known quantities of citric acid, six centrifuge tubes containing 1 to 5 cc. of solution containing 2 mg. per cc. make a convenient series. To each is added 1 cc. of 10 per cent barium acetate solution and four drops of saturated barium hydroxide. The precipitation of the barium salt is completed by the addition of two volumes of 95 per cent alcohol. After standing 10 minutes, the precipitate is centrifuged for 5 minutes and is washed three times by centrifuging with 3 cc. of 50 per cent alcohol containing 1 per cent barium acetate. After the precipitate is well drained (10 minutes at 30° C.), it is dissolved in a mixture of 3 cc. of water and 0.16 cc. of 85 per cent phosphoric acid. This solution is placed in a boiling water bath for 5 minutes to insure complete elimination of the alcohol.

To the hot solution is added 1 cc. of the mercuric sulfate reagent. After the precipitate has settled, the solution is diluted with water to 10 cc., centrifuged, and decanted through a filter into a small beaker. Eight cc. of the filtrate is transferred to a weighed 15 cc. centrifuge tube, and 0.2 cc. of mercuric sulfate reagent and 1 cc. of water are added. The tube is placed for 1 minute in a water bath at 85° C., a drop of 3 per cent hydrogen peroxide is added, and with continuous stirring, 1 per cent potassium permanganate is added at a rate not exceeding 1 drop in 10 seconds until a faint pink color persists for 10 seconds. 1 drop of hydrogen peroxide is added. After 1 minute the stirrer is rinsed off, and the tube cooled and centrifuged for 5 minutes. Upon decanting the supernatant liquid, the precipitate is stirred with 3 cc. of 50 per cent alcohol and centrifuged again. Washing is repeated 3 times. During the process a slight scum occasionally escapes from precipitation; but the combined scum from eight such tubes weighs less than 0.2 mg. After draining for

5 minutes, the tube is wiped off and dried at 100° C. for an hour, or in a vacuum oven for half an hour.

The relation between the mercuric sulfate complex and citric acid may be taken from the following table.

TABLE 36. CITRIC ACID—MERCURIC SULFATE COMPLEX

mg. Citric Acid, Anhydrous	mg. Mercuric Sulfate Complex
2	4
3	8
4	12
5	16
6	20
7	24
8	28

It is apparent that there is a straight line relationship between the mg. of anhydrous citric acid and mg. of mercuric sulfate complex. However, since this relationship is empirical the directions for the determination must be rigidly adhered to, in order to obtain proper results. The weight of the mercuric sulfate complex varies to 1 mg. above the figures quoted above 12 and to 1 mg. below the figures quoted below 12.

No interference is encountered from formic, acetic, succinic, malic, lactic, or tartaric acids present in amounts comparable with the citric acid present. Where much larger amounts of substances precipitable by barium acetate are present, it is necessary to use larger amounts of the reagent. The supernatant liquid from the precipitation of an unknown should be tested with more reagent before discarding. Aconitic acid interferes in this method.

In order to make the method more easily suitable for food products, which might contain interfering substances such as sugars, the citric acid is first separated from the sugars by precipitation with calcium hydroxide. The precipitated calcium citrate is recovered and subsequently analyzed.[21]

Make the sample alkaline with lime water and add an equal volume of alcohol. Centrifuge. Wash the precipitate with 50 per cent alcohol, heat with 85 per cent phosphoric acid and then analyze the solution for citrate according to the method detailed above. This treatment avoids any possible interference from sugars or non-acidic substances.

[21] Bruce, personal communication (1937).

Tartaric Acid

This method developed by Kling[22] and modified by King[23] depends on the insolubility of calcium racemate in dilute acetic and hydrochloric acid.

Preparation of Sample: In jams, jellies and other type products that contain much sugar, pectin or gelatin or other alcohol insoluble material, add sufficient alcohol and a few drops sulfuric acid to a known portion of the sample to precipitate those materials. Allow the mixture to stand until the precipitate has settled and filter off an aliquot portion through a coarse fluted paper or pad of cotton wool. Where esters of tartaric acid may have been formed it may be necessary to saponify before proceeding with the determination. Other samples such as cider may be treated directly.

Reagents: a) Diammonium citrate. Dissolve 29 g. of citric acid in about 200 cc. of water, carefully neutralize to methyl red with ammonia, add 14.5 g. of citric acid, and make up to 1 liter with water. This solution contains 50 g. per liter.

b) Ammonium *l*-tartrate. Dissolve 3.2 g. of the salt, entirely free from *d*-tartrate, in water, add 1 cc. of commercial formalin as a preservative, and dilute to 200 cc.

c) Calcium acetate. Dissolve 16 g. of calcium carbonate in 120 cc. of glacial acetic acid diluted with sufficient water, make up to 1 liter and filter.

d) Hydrochloric acid. Dilute 34 cc. of pure acid to 1 liter.

e) Calcium and sodium acetate. Dissolve 5 g. of calcium carbonate in 20 g. of acetic acid and sufficient water, add 100 g. of sodium acetate, make up the solution to 1 liter and filter.

f) Potassium permanganate. Prepare a solution in water containing 6.974 g. in a liter. Standardize this reagent against pure tartaric acid, employing the complete precipitation process as for the sample taken. One cc. of the potassium permanganate solution equals nearly 2.5 mg. of the *d*-tartaric acid originally present or nearly 5 mg. of racemic acid.

g) Oxalic Acid. Prepare a solution containing 13.879 g. per liter and titrate against the permanganate solution.

Determination: Weigh or measure such a portion of the sample as will contain not more than 0.2 g. of tartaric acid in the final aliquot portion, adjust to 35 cc. by dilution or concentration, add 3 cc. of *N*

[22] Kling, *Ann. fals.* **14**, 185 (1911).

[23] King, *Analyst* **58**, 135 (1933).

sulfuric acid, pour into a 250 cc. flask, rinse with 15 cc. of warm water and then with 95 per cent alcohol, and make up to the mark with the 95 per cent alcohol. Shake the mixture, and allow it to stand for half an hour, filtering if necessary. Transfer a convenient aliquot portion of the clear alcoholic solution so obtained into a centrifuge tube, and add a slight excess of neutral lead acetate solution. Shake vigorously for two minutes, and centrifuge for 15 minutes at about 1000 R.P.M. Drain off the supernatant liquid thoroughly, and wash once with alcohol, centrifuging and draining as before. Transfer the lead salts to a beaker with warm water, and pass in a rapid stream of hydrogen sulfide until the reaction is complete. Filter, wash thoroughly, boil the filtrate until it is free from hydrogen sulfide, and adjust the volume to 150 cc. Up to this point it has been assumed that the difficulties mentioned in the preparation of the sample have been encountered; if they have not, take a portion of the sample, which will contain not more than 0.2 g. tartaric acid, and dilute to 150 cc.

To the 150 cc. obtained by either process, add 15 cc. of diammonium citrate reagent, 25 cc. of ammonium *l*-tartrate reagent and 20 cc. of calcium acetate reagent, stir vigorously until calcium racemate begins to precipitate, and allow the mixture to stand overnight at room temperature. Filter by decantation on to a thin, lightly-tamped pad of asbestos, and transfer the precipitate to the crucible with a portion of the filtrate. Wash the contents of the crucible 5 times with water, filling the crucible about half full and sucking dry each time. Treat the precipitate and mat after removal from the Gooch with 20 cc. of hydrochloric acid reagent and wash the crucible thoroughly. Adjust the volume of solution to 150 cc. with water. Bring 50 cc. of the calcium and sodium acetate reagent to the boiling point and pour it through the Gooch crucible into the 150 cc. mentioned above, then bringing the temperature of the whole to 80° C.; cool, stir vigorously, and leave for at least 4 hours, stirring occasionally. Filter and wash as described in the first operation. Transfer the pad and precipitate to a beaker with 150 cc. of water, add 50 cc. of sulfuric acid 10 per cent by volume and heat to 80° C. Immediately add standardized potassium permanganate solution until an excess is indicated. Again heat to 80° C. add an additional 5 cc. of the potassium permanganate solution and allow the beaker to stand for about 1 minute. After re-heating to 80° C. immediately add 10 cc. of the standardized oxalic acid solution, and titrate back with the potassium permanganate solution. One cc. of the potassium permanganate solution equals 2.5 mg. of *d*-tartaric acid.

Tartaric Acid

The well known Fenton [24] reaction in which a violet color is produced by a tartrate in the presence of ferrous sulfate, hydrogen peroxide and sodium hydroxide may be made the basis for a colorimetric method. This color reaction appears to be specific for tartaric acid. Fenton found that citric acid, succinic, malic, and oxalic acid and sugars do not give the test. The following method, developed by Anderson, Rouse, and Letonoff [25] may be used to determine tartaric acid in tartrate baking powders with or without aluminium.

Reagents: 1) 1 per cent ferrous sulfate. Dissolve 1 g. of ferrous sulfate in 80 cc. of water, heating gently and stirring to aid solution. Cool, transfer to a 100 cc. volumetric flask, and make up to volume.

2) Transfer 16 g. of dry *d*-tartaric acid to a 100 cc. volumetric flask, dissolve in water and make up to volume.

3) Working Tartaric Acid Standard Solution: Transfer 5 cc. of solution (2) to a 100 cc. volumetric flask, add 10.66 cc. of *N* sodium hydroxide solution and make up to volume. This solution contains 0.80 g. of tartaric acid per 100 cc. and has a pH of 6.2.

Determination: Transfer to a small beaker a 2 g. sample of baking powder. Add water drop by drop, until carbon dioxide ceases to be evolved. Next add 45 cc. of water, and stir thoroughly to dissolve the tartrates present. To remove the starch, filter into a 100 cc. volumetric flask and wash the residue 3 times with 15 cc. water at each washing. Make up to volume with water. This solution should have a pH of approximately 6.2. If the pH varies from 6.2 by more than ±0.5, another sample should be prepared and the pH adjusted before making up to volume. The pH of the solution may be determined colorimetrically, using chlorophenol red as an indicator. As a rule tartrate baking powders require no adjustment.

Transfer 10 cc. of the above solution to a 25 cc. volumetric flask. Add 0.2 cc. of 1 per cent ferrous sulfate solution and 0.2 cc. of commercial 3 per cent hydrogen peroxide solution and mix thoroughly. Upon the addition of the hydrogen peroxide, the solution will turn yellow. Allow the solution to stand until it becomes brownish in color and then place it in an ice bath until the brown color disappears and the color becomes definitely lavender. Add immediately 5 cc. of *N* sodium hydroxide solution. Stopper the flask, mix by inversion twice, and place the flask in

[24] Fenton, *Chem. News* 33, 190 (1876).

[25] Anderson, Rouse and Letonoff, *Ind. Eng. Chem., Anal. Ed.* 5, 19 (1933).

the ice bath for 10 minutes. At the end of this time remove the flask from the ice bath, mix by inversion twice, and compare in a colorimeter with a standard prepared simultaneously. For the standard, 10 cc. of the working tartaric acid standard solution containing 0.08 g. of tartaric acid is used. The results may be calculated:

$$\frac{\text{Reading of standard} \times 0.08 \times 10 \times 100}{\text{Reading of unknown} \times \text{weight of sample}} = \% \text{ tartaric acid}$$

VEGETABLE PRODUCTS

The analysis of these products, that is, the estimation of moisture, ash, metallic constituents, sugars, acids, volatile acids, preservatives, and coloring matters are made in a manner entirely similar to other foods and as described in various sections of the text. Chlorides may be determined by the method described under the section, "Fish," Chapter XV. The specific gravity of tomato products may be ascertained by the specific

TABLE 37. PROXIMATE COMPOSITION OF VEGETABLES [27]

Type	Moisture	Protein	Fat	Ash	Carbohydrate
Artichokes[28]	79.5	2.6	0.2	1.0	16.7
Asparagus[28]	94.0	1.8	0.2	0.7	3.3
Beans[28], dried	12.6	22.5	1.8	3.5	59.6
String beans[29]	89.2	2.3	0.3	0.8	7.4
Beets[29]	87.5	1.6	0.1	1.1	9.7
Cabbage[29]	91.5	1.6	0.3	1.0	5.6
Carrots[29]	92.3	1.8	0.5	0.7	4.7
Celery[29]	94.5	1.1	0.1	1.0	3.3
Corn[29]	75.4	3.1	1.1	0.7	19.7
Cucumbers[29]	95.4	0.8	0.2	0.5	3.1
Lettuce[29]	94.7	1.2	0.3	0.9	2.9
Mushrooms[28]	88.1	3.5	0.4	1.2	6.8
Onions[29]	87.6	1.6	0.3	0.6	9.9
Parsnip[29]	83.0	1.6	0.5	1.4	13.5
Peas, dried[28]	9.5	24.6	1.0	2.9	62.0
Peas, green[29]	74.6	7.0	0.5	1.0	16.9
Potatoes[29]	78.3	2.2	0.1	1.0	18.4
Sweet potatoes[29]	69.0	1.8	0.7	1.1	27.4
Radishes[29]	91.8	1.3	0.1	1.0	5.8
Spinach[28]	92.3	2.1	0.3	2.1	3.2
Squash[29]	88.3	1.4	0.5	0.8	9.0
Tomatoes[28]	94.3	0.9	0.4	0.5	3.9
Turnips[29]	89.6	1.3	0.2	0.8	8.1

27 Atwater and Bryant, U. S. Dept. Agr., Bull. No. 28 (1906).

28 As purchased.

29 Edible portion.

Table 38. Proximate Composition of Fresh Vegetables. Edible Portion [30]

Type	Water %	Protein %	Fat %	Ash %	Total Carbo[31]	Fiber	Sugars	Starch
Asparagus...	ave. 93.0	2.2	0.2	0.67	3.9	0.7	1.34	0.4
	max. 94.4	3.4	0.3	0.97	....	0.9	2.96	0.7
	min. 90.8	1.1	0.0	0.49	....	0.7	0.59	0.1
Beets.......	ave. 87.6	1.6	0.1	1.11	9.6	0.9	....	....
	max. 94.1	2.2	0.3	2.0	....	1.7	....	....
	min. 82.3	0.9	0.0	0.7	....	0.6	....	....
Cabbage....	ave. 92.4	1.4	0.2	0.75	5.3	1.0	3.5	....
	max. 94.8	3.1	0.5	1.07	....	1.4	4.8	....
	min. 88.4	0.8	0.1	0.34	....	0.5	2.9	....
Carrots......	ave. 88.2	1.2	0.3	1.02	9.3	1.1	7.5	....
	max. 91.1	2.3	0.7	1.55	....	2.3	8.7	....
	min. 83.1	0.7	0.0	0.62	....	0.7	6.2	....
Sweet Corn..	ave. 73.9	3.7	1.2	0.66	20.5	0.8	4.29	14.6
	max. 86.1	4.9	2.1	0.84	....	1.4	7.56	26.2
	min. 61.3	2.8	0.5	0.4	....	0.5	1.58	3.4
Jerusalem Artichoke .	ave. 79.5	2.2	0.1	1.17	17.0[32]	0.8	....	....
	max. 84.2	3.1	0.2	2.0	....	1.4	....	....
	min. 74.2	1.1	0.0	0.87	....	0.6	....	....
Lettuce.....	ave. 94.8	1.2	0.2	0.91	2.9	0.6	1.6	....
	max. 97.4	1.9	0.6	1.41	....	1.1	2.2	....
	min. 91.5	0.5	0.0	0.5		0.3	0.9	....
Mushrooms..	ave. 91.1	(0.57)[33]	0.3	1.14	....	0.9	....	....
	max. 94.7	(0.98)	0.8	1.86	....	1.3	....	....
	min. 87.9	(0.27)	0.1	0.58	....	0.2	....	....
Onions......	ave. 87.5	1.4	0.2	0.58	10.3	0.8	6.7	0.5
	max. 95.2	2.7	0.8	1.20	....	1.8	8.4	....
	min. 70.2	0.4	0.1	0.17	....	0.4	3.7	....
Parsnips.....	ave. 78.6	1.5	0.5	1.15	18.2	2.2	9.5	2.4
	max. 89.2	2.1	0.8	1.9	....	3.0	14.2	8.0
	min. 72.6	1.1	0.2	0.7	....	1.4	4.5	0.0
Peas.......	ave. 74.3	6.7	0.4	0.92	17.7	2.2	3.2	8.2
	max. 84.1	9.9	0.6	1.2	....	2.9	6.9	15.9
	min. 56.7	3.5	0.1	0.55	....	1.3	0.4	1.8
Potatoes....	ave. 77.8	2.0	0.1	0.99	19.1	0.4	0.87	14.7
	max. 85.2	3.9	0.3	1.9	....	0.9	1.5	16.4
	min. 66.0	0.9	0.0	0.5	....	0.2	0.21	12.1
Spinach.....	ave. 92.7	2.3	0.3	1.53	3.2	0.6	0.3	....
	max. 95.0	3.4	0.6	2.0	....	0.7	0.4	....
	min. 89.4	1.9	0.1	1.06	....	0.5	0.2	....
Sweet Potato	ave. 68.5	1.8	0.7	1.07	27.9	1.0	5.35	20.2
	max. 82.7	4.4	2.5	1.85	....	1.8	11.9	29.8
	min. 58.5	0.5	0.2	0.4	....	0.6	1.15	8.8
Tomatoes ...	ave. 94.1	1.0	0.3	0.57	4.0	0.6	3.37	....
	max. 96.7	1.8	0.5	1.0	....	1.2	4.06	....
	min. 90.6	0.7	0.1	0.34	....	0.2	2.3	....
Turnips.....	ave. 90.9	1.1	0.2	0.73	7.1	1.1	4.6	
	max. 95.7	2.1	0.4	1.0	....	1.4	....	
	min. 85.6	0.7	0.1	0.5	....	0.6	....	

[30] Chatfield and Adams, U. S. Dept. Agr., Circ. No. 146 (1931).

[31] Total carbohydrate, by difference. [32] Mostly inulin. [33] As nitrogen, protein cannot be calculated from nitrogen because of much non-protein nitrogen.

gravity centrifuge bottle method detailed in Chapter 1. Organoleptic examination should be made as directed in Chapter 1.

The composition, by means of promixate analysis, of some vegetables is given in Tables 37 and 38. An extended survey of the chemical composition not only of vegetables but also of fruits and nuts is given by McCance, Widdowson and Shackleton.[34]

REFERENCES

Brooks, "Critical Studies in the Legal Chemistry of Foods," Chemical Catalog (1927).

Methods of A. O. A. C. (1935).

Allen's Commercial Organic Analysis, 5th ed., Vol. X, Blakiston (1933).

Cox, "Analysis of Foods," Blakiston (1926).

Hinton, Analyst, 59, 248 (1934).

McCance, Widdowson, and Shackleton, "The Nutritive Value of Fruits, Vegetables and Nuts," Med. Research Council, Special Rep. Ser., No. 213 (1936).

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[34] McCance, Widdowson and Shackleton, Med. Research Council, Special Rep. Ser. No. 213 (1936).

CHAPTER XII

SPICES, FLAVORS AND CONDIMENTS

SPICES are aromatic vegetable substances used for the seasoning of food. They are true to name, and from them no portion of any volatile oil or other flavoring principle has been removed. In Table 39 are tabulated the standards promulgated by the Food and Drug Administration. In general, some of the determinations of spices follow closely those methods we have previously encountered. Thus moisture, ash, nitrogen, copper reducing materials, and starch may be estimated as described in other sections of the text. Care must be taken in grinding the samples so that a uniform mixture results. If possible, the spice should be ground to an impalpable powder. Tables 40 and 41 contain analyses of genuine spices.

EXTRACTS

The volatile and non-volatile ether extract may be ascertained by extracting 2 g. of the ground sample in a continuous extractor for 20 hours with anhydrous ether. Evaporate the ether to a small volume. Transfer to a small weighed beaker or micro-beaker, taking care to wash out the original flask with small portions of anhydrous ether. Evaporate at room temperature and allow to stand for 18 hours over sulfuric acid in a desiccator. Weigh and calculate the gain in weight as percentage of total ether extract. Heat the extract gradually and then at 110° C. until successive weighings show only a small loss. The difference in weight equals the volatile ether extract. The residue is the non-volatile ether extract.

The alcohol extract is determined by shaking, at intervals, 2 g. of the sample in a 100 cc. volumetric flask with 95 per cent alcohol for 8 hrs. Filter, and evaporate a 50 cc. aliquot to dryness in a tared dish. Dry at 100° C. The residue is the alcohol extract.

TABLE 39. STANDARDS FOR SPICES [1]

Allspice, pimento	The dried, nearly ripe fruit of *Pimenta officinalis* Lindl. It contains not less than 8 per cent. of quercitannic acid, calculated from the total oxygen absorbed by the aqueous extract, not more than 25 per cent. of crude fiber, not more than 6 per cent. of total ash, nor more than 0.4 per cent. of ash insoluble in hydrochloric acid

[1] Service and Regulatory Announcements, Food and Drug No. 2, U. S. Dept. Agr., Food and Drug Admin. (1936).

Anise, Aniseed	The dried fruit of *Pimpinella anisum* L. It contains not more than 9 per cent. of total ash, nor more than 1.5 per cent. of ash insoluble in hydrochloric acid.
Caraway, Caraway seed	The dried fruit of *Carum carvi* L. It contains not more than 8 per cent. of total ash, nor more than 1.5 per cent. of ash insoluble in hydrochloric acid.
Cardamom seed	The dried seed of cardamom, *Elettaria cardmomum* Maton. It contains not more than 8 per cent. of total ash, nor more than 3 per cent. of ash insoluble in hydrochloric acid.
Red Pepper	The red, dried, ripe fruit of any species of *Capsicum*. It contains not more than 8 per cent. of total ash, nor more than 1 per cent. of ash insoluble in hydrochloric acid.
Cayenne Pepper, Cayenne	The dried, ripe fruit of *Capsicum frutescens* L., *C. baccatum* L., or some other small-fruited species of *Capsicum*. It contains not less than 15 per cent. of nonvolatile ether extract, not more than 1.5 per cent. starch, not more than 28 per cent. crude fiber, not more than 8 per cent. of total ash, nor more than 1.25 per cent. of ash insoluble in hydrochloric acid.
Paprika	The dried ripe fruit of *Capsicum annuum* L. It contains not more than 8.5 per cent. of total ash, nor more than 1 per cent. of ash insoluble in hydrochloric acid. The iodine number of its extracted oil is not less than 125, nor more than 136.
Hungarian Paprika	The paprika having the pungency and flavor characteristic of that grown in Hungary. (a) Rosenpaprika, rosapaprika, rose paprika, is Hungarian paprika prepared by grinding specially selected pods of paprika, from which the placentae, stalks, and stems have been removed. It contains no more seeds than in normal pods, not more than 18 per cent. of nonvolatile ether extract, not more than 23 per cent. of crude fiber, not more than 6 per cent. of total ash, nor more than 0.4 per cent. of ash insoluble in hydrochloric acid. (b) Koenigspaprika, king's paprika, is Hungarian paprika prepared by grinding whole pods of paprika without selection, and includes the seeds and stems naturally occurring with the pods. It contains not more than 18 per cent. nonvolatile ether extract, not more than 23 per cent. crude fiber, nor more than 6.5 per cent. of total ash, nor more than 0.5 per cent. of ash insoluble in hydrochloric acid.
Pimenton, Pimiento, Spanish Paprika	Paprika having the characteristics of that grown in Spain. It contains not more than 18 per cent. of nonvolatile ether extract, not more than 21 per cent. of crude fiber, not more than 8.5 per cent. of total ash, nor more than 1 per cent. of ash insoluble in hydrochloric acid.
Celery seed	The dried fruit of *Celeri graveolens* (L.) Britton (*Apium graveolens* L.). It contains not more than 10 per cent. of total ash, nor more than 2 per cent. of ash insoluble in hydrochloric acid.
Ground Cinnamon, Ground Cassia	The powder made from cinnamon, the dried bark of cultivated varieties of *Cinnamomum zeylanicum* Nees or of *C. cassia* (L.) Blume. It contains not more than 5 per cent. of total ash, nor more than 2 per cent. of ash insoluble in hydrochloric acid.
Cloves	The dried flower buds of *Caryophyllus aromaticus* L. They contain not more than 5 per cent. of clove stems, not less than 15 per cent. of volatile ether extract, not less than 12 per cent. of quercitannic acid, calculated from the total oxygen absorbed by the aqueous extract, not more than 10 per cent. of crude fiber, not more than 7 per cent. of total ash, nor more than 0.5 per cent. of ash insoluble in hydrochloric acid.
Coriander seed	The dried fruit of *Coriandrum sativam* L. It contains not more than 7 per cent. of total ash, nor more than 1.5 per cent. of ash insoluble in hydrochloric acid.

Cumin seed	The dried fruit of *Cuminum cyminum* L. It contains not more than 9.5 per cent. of total ash nor more than 1.5 per cent. of ash insoluble in hydrochloric acid, nor more than 5 per cent. of harmless foreign matter.
Dill seed	The dried fruit of *Anethum graveolens* L. It contains not more than 10 per cent. of total ash, nor more than 3 per cent. of ash insoluble in hydrochloric acid.
Fennel seed	The dried fruit of cultivated varieties of *Foeniculum vulgare* Hill. It contains not more than 9 per cent. of total ash nor more than 2 per cent. of ash insoluble in hydrochloric acid.
Ginger	The washed and dried, or decorticated and dried, rhizome of *Zingiber officinale* Roscoe. It contains not more than 8 per cent. of crude fiber, not less than 42 per cent. of starch, not more than 1 per cent. of lime, calcium oxide, not less than 12 per cent. of cold water extract, not more than 7 per cent. of total ash, nor more than 2 per cent. of ash insoluble in hydrochloric acid, nor less than 2 per cent. of ash soluble in cold water.
Mace	The dried arillus of *Myristica fragrans* Houtt. It contains not less than 20 per cent. nor more than 30 per cent. of nonvolatile ether extract, not more than 10 per cent. of crude fiber, not more than 3 per cent. of total ash, nor more than 0.5 per cent. ash insoluble in hydrochloric acid.
Marjoram, Leaf Marjoram	The dried leaves, with or without a small proportion of the flowering tops, of *Majorana hortensis* Moench. It contains not more than 16 per cent. of total ash, not more than 4.5 per cent. of ash insoluble in hydrochloric acid, nor more than 10 per cent. of stems and harmless foreign material.
Mustard seed	The seed of *Sinapis alba* L., white mustard, *Brassica nigra* (L.) Koch, black mustard, *B. juncea* (L.) Cosson, or varieties or closely related species of the types *B. nigra* and *B. juncea*. White mustard contains no appreciable amount of volatile oil. It contains not more than 5 per cent. of total ash nor more than 1.5 per cent. of ash insoluble in hydrochloric acid. *Brassica nigra*, black mustard, and *B. juncea* yield 0.6 per cent. of volatile mustard oil, calculated as allylisothiocyanate. The varieties and species closely related to the types of *B. nigra* and *B. juncea* yield not less than 0.6 per cent. of volatile mustard oil, similar in character and composition to the volatile oils yielded by *B. nigra* and *B. juncea*. These mustard seeds contain not more than 5 per cent. of total ash, nor more than 1.5 per cent. of ash insoluble in hydrochloric acid.
Nutmeg	The dried seed of *Myristica fragrans* Houtt deprived of its testa, with or without a thin coating of lime, calcium oxide. It contains not less than 25 per cent. of nonvolatile ether extract, not more than 10 per cent. of crude fiber, not more than 5 per cent. of total ash, nor more than 0.5 per cent. of ash insoluble in hydrochloric acid.
Black Pepper	The dried immature berry of *Piper nigrum* L. It contains not less than 6.75 per cent. of nonvolatile ether extract, not less than 30 per cent. starch, not more than 7 per cent. of total ash, not more than 1.5 per cent. of ash insoluble in hydrochloric acid.
White Pepper	The dried mature berry of *Piper nigrum* L. from which the outer coating, or the outer and inner coatings, have been removed. It contains not less than 52 per cent. of starch, not less than 7 per cent. of nonvolatile ether extract, not more than 5 per cent. of crude fiber, not more than 3.5 per cent. of total ash, nor more than 0.3 per cent. of ash insoluble in hydrochloric acid.
Saffron	The dried stigma of *Crocus sativus* L. It contains not more than 10 per cent. of yellow styles and other foreign matter, not more

than 14 per cent. of volatile matter when dried at 100° C., not more than 7.5 per cent. of total ash, nor more than 1 per cent. of ash insoluble in hydrochloric acid.

Thyme — The dried leaves and flowering tops of *Thymus vulgaris* L. It contains not more than 14 per cent. of total ash, nor more than 4 per cent. of ash insoluble in hydrochloric acid.

Star Aniseed — The dried fruit of *Illicium verum* Hook. It contains not more than 5 per cent. of total ash.

TABLE 40. ANALYSES OF GENUINE PEPPER [2]

Type		Moisture %	Non-volatile ether extract %	Crude fiber %	Dextrose %	Alcohol extract %	Nitrogen %
Lampong	max.	12.38	10.74	13.70	51.32	12.26	2.28
	min.	8.73	7.30	11.76	44.28	10.22	1.84
	ave.	10.06	9.29	12.50	47.20	11.20	2.09
Alleppi	max.	10.86	10.46	13.02	51.76	14.34	2.22
	min.	9.80	7.97	10.85	47.48	10.00	1.95
	ave.	10.13	8.95	11.66	50.36	11.15	2.08
Tellicherry	max.	10.27	9.37	14.36	53.36	11.20	2.16
	min.	8.20	7.25	11.56	51.20	9.60	1.97
	ave.	9.34	8.31	13.08	51.90	10.35	2.06
White and decorticated	max.	11.65	9.70	4.86	76.56	9.36	2.02
	min.	10.34	6.18	1.03	64.88	7.38	1.56
	ave.	11.05	7.79	3.64	68.64	8.50	1.89

ASH

Type		Total %	Water soluble %	Water insoluble %	Acid insoluble %	Alkalinity of soluble[3]	Alkalinity of insoluble[3]
Lampong	max.	6.29	2.97	3.88	1.02	2.9	4.8
	min.	4.39	1.98	1.99	0.11	2.3	2.5
	ave.	5.05	2.33	2.72	0.41	2.6	4.1
Alleppi	max.	5.83	3.61	2.66	0.36	3.0	3.9
	min.	4.18	2.50	1.70	0.02	2.4	2.3
	ave.	4.74	2.88	1.86	0.11	2.6	3.3
Tellicherry	max.	4.75	2.87	1.99	0.11	2.6	3.5
	min.	4.41	2.54	1.71	0.07	2.4	2.4
	ave.	4.55	2.71	1.84	0.08	2.5	3.0
White and decorticated	max.	4.84	0.50	4.34	1.28	0.3	2.2
	min.	0.83	0.07	0.76	0.05	0.1	0.9
	ave.	2.01	0.23	1.78	0.37	0.2	1.5

[2] Smith, Alfend and Mitchell, *J. Assoc. Official Agr. Chem.* 9, 340 (1926).

[3] Alkalinity of water soluble and insoluble ash in cc. 0.1 *N* acid per 1 g.

Table 41. Analyses of Pure Spices [4]

		Moisture %	Total ash %	Water soluble ash %	Ash insoluble in HCl %	Volatile ether extract %	Non-volatile ether extract %	Alcohol extract %
Black pepper	max.	12.95	6.52	3.20	1.19	1.60	10.37	11.86
	min.	10.63	3.04	1.75	0.00	0.65	6.86	8.47
	ave.	11.96	4.76	2.51	0.47	1.14	8.42	9.62
White pepper	max.	14.47	2.96	0.80	0.20	0.95	7.94	8.55
	min.	12.72	1.03	0.28	0.00	0.49	6.26	7.19
	ave.	13.41	1.77	0.47	0.10	0.73	6.91	7.66
Cayenne pepper	max.	7.08	5.96	4.93	0.23	2.57	21.81	27.61
	min.	3.67	5.08	3.30	0.05	0.73	17.17	21.52
	ave.	5.73	5.43	3.98	0.15	1.35	20.15	24.35
Ginger	max.	11.72	9.35	4.09	2.29	3.09	5.42	6.58
	min.	8.71	3.61	1.73	0.02	0.96	2.82	3.63
	ave.	10.44	5.27	2.71	0.44	1.97	4.10	5.18
Cinnamon	max.	10.48	5.99	2.71	0.58	1.62	1.68	13.60
	min.	7.79	4.16	1.40	0.02	0.72	1.35	9.97
	ave.	8.63	4.82	1.87	0.13	1.39	1.44	12.21
Cassia	max.	11.91	6.20	2.52	2.42	5.15	4.13	16.74
	min.	6.53	3.01	0.71	0.02	0.93	1.32	4.57
	ave.	4.24	4.73	1.68	0.56	2.61	2.12	8.29
Cloves	max.	8.26	6.22	3.75	0.13	20.53	6.67	15.58
	min.	7.03	5.28	3.25	0.00	17.82	6.24	13.99
	ave.	7.81	5.92	3.58	0.06	19.18	6.49	14.87
Allspice or pimento	max.	10.14	4.76	2.69	0.06	5.21	7.72	14.27
	min.	9.45	4.15	2.29	0.00	3.38	4.35	7.39
	ave.	9.78	4.47	2.47	0.03	4.05	5.84	11.79
Nutmegs	max.	10.83	3.26	1.46	0.01	6.94	36.94	17.38
	min.	5.79	2.13	0.82	0.00	2.56	28.73	10.42

[4] Winton, Ogden and Mitchell, Conn. Agr. Exptl. Sta. Ann. Rep. (1898).

TABLE 41. ANALYSES OF PURE SPICES [4]—*Continued*

		Reducing matters* %	Starch by diastase %	Crude fiber %	Protein %	Total nitrogen %	Cold water extract %	Lime %
Black pepper	max.	43.47	39.66	18.25	13.81†	2.53	....	....
	min.	28.15	22.05	10.75	10.50	2.03	....	....
	ave.	38.63	34.15	13.06	12.05	2.26	....	....
White pepper	max.	64.92	63.60	4.25	11.19†	2.13	....	....
	min.	56.43	53.11	0.54	10.44	1.95	....	....
	ave.	59.17	56.47	3.14	10.89	2.04	....	....
Cyenne pepper	max.	9.31	1.46	24.91	14.63	2.34	....	....
	min.	7.15	0.80	20.35	13.31	2.13	....	....
	ave.	8.47	1.01	22.35	13.67	2.18	....	....
Ginger	max.	62.42	60.31	5.50	9.75	1.45	17.55	3.53
	min.	53.43	49.05	2.37	4.81	0.77	10.92	0.20
	ave.	57.45	54.53	3.91	7.74	1.23	13.42	0.80
							Oxygen absorbed by aqueous extract	Quercitannic acid equivalent to oxygen absorbed
Cinnamon	max.	22.00	....	38.48	4.06	0.65		
	min.	16.65	....	34.38	3.25	0.52		
	ave.	19.30	....	36.20	3.70	0.59		
Cassia	max.	32.04	....	28.80	5.44	0.87		
	min.	16.65	....	17.03	3.31	0.53		
	ave.	23.32	....	22.96	4.34	0.69		
Cloves	max.	9.63	3.15	9.02	7.06	1.13	2.63	20.54
	min.	8.19	2.08	7.06	5.88	0.94	2.08	16.25
	ave.	8.99	2.74	8.10	6.18	0.99	2.33	18.19
Allspice or pimento	max.	20.65	3.76	23.98	6.37	1.02	1.59	12.48
	min.	16.56	1.82	20.46	5.19	0.83	1.03	8.06
	ave.	18.03	3.04	22.39	5.75	0.92	1.24	9.71
Nutmegs	max.	25.60	24.20	3.72	7.00	1.12	....	....
	min.	17.19	14.62	2.38	6.56	1.05	....	....

*By direct inversion, calculated as starch.

†Total nitrogen less nitrogen in non-volatile ether extract × 6.25.

[4] Winton, Ogden and Mitchell, Conn. Agr. Exptl. Sta. Ann. Rep. (1898).

Volatile Oil

The volatile oil is that oil that can be obtained from a spice by distillation. The methods of the A. O. A. C. give the following details. Transfer a weighed quantity of whole or ground material to a 500–2000 cc. round-bottomed, short-necked flask, in amount sufficient to yield, if possible, 2 cc. or more of volatile oil. Add to the flask 3–6 times as much water as material, and mix uniformly. Set up an apparatus[5] consisting of the flask, an appropriate oil separatory trap, Fig. 51, and a tube condenser extending into the trap, Fig. 52. With an oil bath (hydrogenated cottonseed oil is satisfactory) as the source of heat, boil the contents of the flask slowly 4–8 hours, or until all the volatile oil has been distilled, taking care to avoid the escape of vapors around the condenser. With spices, for example nutmeg, containing volatile oils lighter than water and also fixed oils heavier than water, discontinue the distillation when the fraction of oil obtained during a 1 hour period is heavier than water.

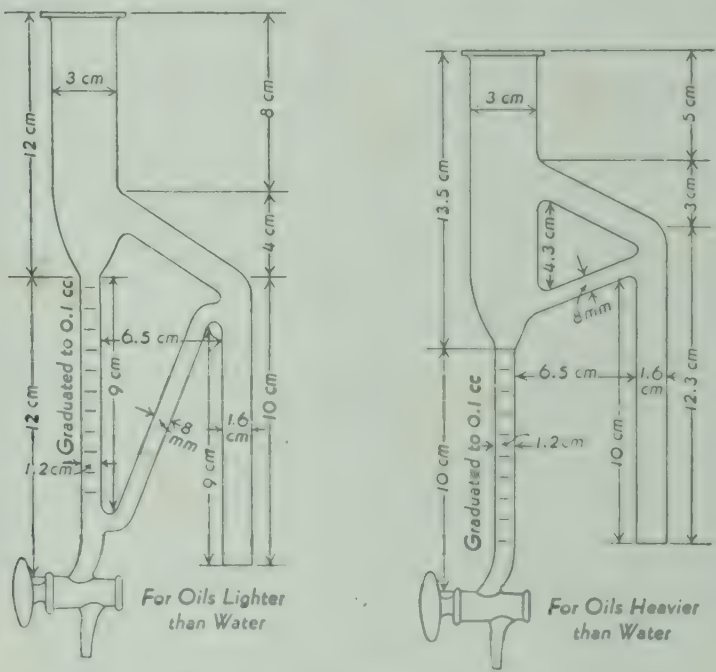

Fig. 51. Oil Separatory Tubes

In case of unsatisfactory separation of the volatile oil, draw off the contents of the trap into a small separatory funnel. After separation,

[5] *J. Assoc. Official Agr. Chem.* 17, 70 (1934).

return the water to the trap and transfer the volatile oil to a graduated cylinder. Repeat the procedure if necessary.

With volatile oil heavier than water, after transferring to the graduated cylinder, run the water with any remaining oil into a small separatory funnel. Wash the oil trap with 10 cc. of ether and transfer the washings to the funnel. Shake, and withdraw the ether. Evaporate the ether and drain the residue into the cylinder. Read the quantity of volatile oil directly in the cylinder and report the oil in terms of cc. per 100 g. of spice.

Determine the physical and chemical constants of the oil in the usual manner, after it has been allowed to stand until perfectly clear or has been dried with a minimum quantity of anhydrous sodium sulfate and allowed to settle. Owing to the small quantity of oil available, great care should be exercised to avoid loss in any of these determinations.

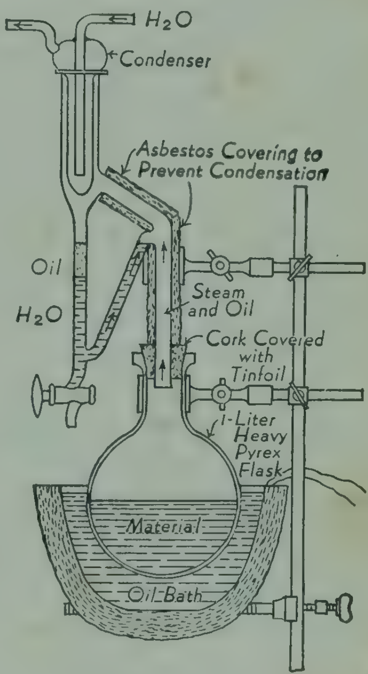

FIG. 52. Volatile Oil Distillation Apparatus

Volatile Oil in Mustard Seed—Place 5 g. of the ground seed (No. 20 powder) in a 200 cc. flask, add 100 cc. water, stopper tightly and macerate for 2 hours at about 37° C. Then add 20 cc. of 95 per cent alcohol and distill about 60 cc. into a 100 cc. volumetric flask containing 10 cc. of ammonium hydroxide (1:2), taking care that the end of the condenser dips below the surface of the solution. Add 20 cc. of 0.1 *N* silver nitrate solution to the distillate, set aside overnight, heat to boiling on a water bath in order to agglomerate the silver sulfide, cool, make up to 100 cc. with water, and filter. Acidify 50 cc. of the filtrate with about 5 cc. nitric acid and titrate with 0.1 *N* ammonium thiocyanate, using 5 cc. of 10 per cent ferric ammonium sulfate solution as an indicator. 1 cc. of 0.1 *N* silver nitrate consumed = 0.004956 g. of allylisothiocyanate.

CRUDE FIBER

By the term "crude fiber" is meant in food analysis the combustible residue which is left after the other carbohydrates and the proteins have

been removed by successive treatments with boiling acid and alkali. This residue is largely cellulose and consists of carbohydrates not assimilable by humans. The method devised by Henneberg has been rigidly standardized by the A. O. A. C.

Reagents: a) Sulfuric acid solution—Contains 1.25 g. of H_2SO_4 per 100 cc.

b) Sodium hydroxide solution—Contains 1.25 g. of NaOH per 100 cc. free, or nearly free from sodium carbonate.

The strength of these solutions must be accurately checked by titration.

c) Asbestos—Digest on a steam bath or at an equivalent temperature for at least 8 hours with an approximately 5 per cent sodium hydroxide solution and thoroughly wash with hot water; then digest in a similar manner for 8 hours with hydrochloric acid (1:3) and again wash thoroughly with hot water. Dry, and ignite at bright red heat.

Apparatus: a) Condenser—Use a condenser that will maintain a constant volume of solution throughout the process of digestion.

b) Digestion flasks—Use digestion flasks of such size and shape that the solution will be not less than 1 inch nor more than 1.5 inch in depth. A 700–750 cc. Erlenmeyer flask is recommended.

c) Filtering cloth—Use filtering cloth of such character that no appreciable solid matter passes through when filtering is rapid. Butchers linen or dress linen with about 45 threads to the inch or No. 40 filtering cloth made by the National Filter Cloth and Weaving Co., or its equivalent, may be used.

Determination: Extract 2 g. of the dry material with ordinary ether, or use the residue from the ether extract determination and transfer the residue, together with about 0.5 g. of asbestos, to the digestion flask. If the residue from the ether extract is used and the proper quantity of asbestos has already been added, further addition is unnecessary. Add 200 cc. of the boiling sulfuric acid solution, immediately connect with the condenser, and heat. It is essential that the contents of the flask come to boiling within 1 minute and that the boiling continue briskly for exactly 30 minutes. Rotate the flask about every 5 minutes in order to mix the charge thoroughly. Take care to keep the material from remaining on the sides of the flask out of contact with the solution. A blast of air conducted into the flask will serve to reduce frothing of the liquid. At the expiration of 30 minutes remove the flask, immediately filter through linen in a fluted funnel, and wash with boiling water until the

washings are no longer acid. Bring a quantity of the sodium hydroxide solution to boiling and keep at this temperature under a reflux condenser until used. Wash the charge and the asbestos back into the flask with 200 cc. of the boiling sodium hydroxide solution, using a wash bottle marked to deliver 200 cc. The boiling sodium hydroxide solution is conveniently transferred to the 200 cc. wash bottle by means of a bent tube through which the liquid is forced by blowing into a tube connected with the top of the reflux condenser attached to the sodium hydroxide flask. Then connect the flask with the reflux condenser and boil for exactly 30 minutes. The boiling with the alkali should be so timed that the contents of the different flasks will reach boiling point approximately 3 minutes apart, which permits sufficient time for filtration. At the expiration of 30 minutes, remove the flask and immediately filter through a Gooch prepared with an asbestos mat, through an alundum crucible, or through the filtering cloth in a fluted funnel. If the filter cloth is used, thoroughly wash the residue with boiling water and then transfer it to a Gooch crucible prepared with a thin but close layer of ignited asbestos. After thorough washing with boiling water, wash with about 15 cc. of 95 per cent alcohol. Dry the crucible and contents at 110° C. to constant weight. Cool in an efficient desiccator and weigh. Incinerate the contents of the crucible in an electric muffle or over a Meker burner at a dull red heat, until the carbonaceous matter has been consumed, which will take about 20 minutes. Cool in a desiccator and weigh. The loss in weight is reported as crude fiber.

Microscopic Examination

A valuable means and in some cases, a better means, of detecting adulteration in spices is microscopic examination. The reader is referred to more specialized texts, such as Winton,[6] for detailed methods of microscopic examination of spices.

MAYONNAISE AND SALAD DRESSINGS

Mayonnaise and salad dressings may in general be analyzed by methods detailed previously. Mayonnaise, mayonnaise dressing, or mayonnaise salad dressing is the semisolid emulsion of edible vegetable

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[6] Winton, "Structure and Composition of Foods," Wiley (1932) (1935) (1937).

oil, egg yolk, or whole egg, a vinegar, and/or lemon juice, with one or more of the following: Salt, other seasoning commonly used in its preparation, sugar, and/or dextrose. The finished product contains not less than 50 per cent of edible vegetable oil.

In recent years many products have been manufactured and sold under the name of salad dressing that do not conform to the definition given above. These products contain much more water and much less oil and eggs and are generally stabilized by the use of gums, starch of one type or another, or some other thickening agent. The presence of such materials may be shown by methods completely detailed in the Chapters on Milk, Milk Products and Gums. Coloring matter may be determined in the usual manner, although a much simpler and more rapid method is to follow the procedure as outlined in the method for gums, by using trichloroacetic acid to break the emulsion. The water soluble colors will be in the clear filtrate and may be taken up directly on wool, and subsequently redyed. The oil soluble colors are in the petroleum ether washings and may be extracted in the usual manner.

The egg content is based on the lipoid-phosphorus pentoxide content, assuming that all of the lipoid-phosphorus pentoxide comes from eggs. This is not necessarily the case and will be discussed in a subsequent section. Lipoid-phosphorus pentoxide may be estimated according to the method detailed in the section "Egg and Egg-products," Chapter XV.

Calculation of Composition

The A. O. A. C. gives the following formulae for the calculation of the composition of mayonnaise salad dressing, and other salad dressings.

When $P = \%$ total P_2O_5 and $N = \%$ total nitrogen, then

$$\%\text{ yolk} = 75.69\,P - 1.802\,N;$$
$$\%\text{ white} = 60.80\,N - 114.59\,P;$$
$$\%\text{ total egg} = \%\text{ yolk} + \%\text{ white};$$

$$\%\text{ white in egg component} = \frac{\%\text{ white}}{\%\text{ total egg}} \times 100$$

$$\text{Vegetable oil} = \text{total fat} - (\text{yolk} \times 0.3188);$$
$$\text{Vinegar (4\% acid strength)} = \text{total acidity as acetic} \times 25$$

Minor constituents

$$\begin{aligned}\text{(sugar, salt, spices, stabilizers)} &= \text{total solids} - (\text{yolk} \times 0.5047) \\ &\quad - (\text{white} \times 0.1221) \\ &\quad - \text{vegetable oil} \\ \text{Added water} &= 100\% - \text{total egg} - \text{vegetable oil} \\ &\quad - \text{vinegar} \\ &\quad - \text{minor constituents.}\end{aligned}$$

The total fat may be estimated by the acid hydrolysis modification of the Roese-Gottlieb method as detailed under "Cheese," Chapter 7. The oil may be identified by the methods outlined in the Chapter on Oils and Fats, Chapter VIII.

Mineral Oil in Mayonnaise and Salad Dressing

A mayonnaise, according to definition, should contain 50 per cent of edible vegetable oil. Some manufacturers have stressed the use of mayonnaise in so-called non-fattening diets. Indeed in certain cases, the edible oil has been replaced in whole or in part by mineral oil in order to substantiate such claims.

Place 50 g. of mayonnaise or salad dressing in a beaker and add 25 cc. of alcohol. Warm on a steam bath until the emulsion is broken and the oil separates sufficiently to float on top. Transfer to a separatory funnel and add 200 cc. of warm water. When the layers separate, run off the water and transfer the oil to a cylinder. After allowing to stand the clear oil may be poured off.

Saponify 2 g. of the clear oil with 15 cc. of an alcoholic solution of potassium hydroxide, 40 g. potassium hydroxide per liter of alcohol, and evaporate off the alcohol on the steam bath. Add 50 cc. of water and dissolve the soaps by heat, if necessary. If mineral oil is present, in comparatively large amounts, a cloudy emulsion will be formed with globules of oil floating on the surface. Normally a slight amount of unsaponifiable matter is present in an oil or fat and a slight cloud may result which should be neglected. If doubtful cases arise, the presence of much unsaponifiable matter may be demonstrated by the quantitative method described in the chapter on oils and fats, Chapter VIII.

FLAVORING EXTRACTS

A flavoring extract is a solution in ethyl alcohol of proper strength of the sapid and odorous principles derived from an aromatic plant, or

parts of the plant, with or without its coloring matter, conforming in name to the plant used in its preparation.

Vanilla Extract

Vanilla extract is the flavoring extract prepared from vanilla bean, with or without one or more of the following: Sugar, dextrose, glycerol. It contains in 100 cc. the soluble matters from not less than 10 g. of the vanilla bean.

Alcohol, glycerol, total solids, ash, sucrose and other sugars, coloring matters, alcohol denaturants and caramel may be detected and estimated as detailed in the respective chapters covering these determinations.

Table 42. Composition of Vanilla Extract [7]

	Maximum	Minimum	Average
Vanillin g. 100 cc.	0.31	0.11	0.19
Lead number	0.74	0.40	0.54
Acidity of extract, cc. 0.1 *N* alkali per 100 cc.			
Total	52	30	42
Equivalent to Vanillin	20	7	12
Other than Vanillin	42	14	30
Ash g./100 cc.			
Total	0.432	0.220	0.319
Water soluble	0.357	0.179	0.265
Water insoluble	0.081	0.027	0.054
Alkalinity of ash, cc. 0.1 *N* acid per 100 cc.			
Total	54	30	42
Water soluble	40	22	30
Water insoluble	42	30	12

Gravimetric Method for Vanillin and Coumarin
(Hess and Prescott method [8])

Transfer by means of a pipette 50 cc. of the extract to a 250 cc. beaker with marks showing volumes at 80 cc. and 50 cc. Dilute the extract to 80 cc. and evaporate to 50 cc. on a water bath kept at 70° C. Dilute again with water to 80 cc. and again evaporate to 50 cc. Transfer to a 100 cc. flask, rinsing the beaker with hot water, add 25 cc. of 8 per cent neutral

[7] Winton, Albright and Berry, *Ind. Eng. Chem.* 7, 516 (1915).

[8] Hess and Prescott, *J. Am. Chem. Soc.* 21, 256 (1899).

lead acetate solution, make up to volume with water, shake and allow to stand overnight at 37° C. in an incubator, or water bath thermostatically controlled or on the outside top of an oven whose inside temperature is 100° C. Filter through a small dry filter, reserving the filtrate for the determination of vanillin and coumarin, and lead number.

Vanillin. Transfer a 50 cc. aliquot by means of a pipette to a Jacobs-Singer separatory flask and extract with four 15 cc. portions of ether washed twice to remove the alcohol. Pour the clear successive ether layers from the orifice of the separatory flask into a separatory funnel. Wash the combined ether layers with 4 or 5 portions of ammonium hydroxide solution (1:11), using 10 cc. the first time and 5 cc. each time after the first. Save the ether layer for the coumarin determination. Slightly acidify the combined ammoniacal solutions with hydrochloric acid (1:2), cool, and transfer to a Jacobs-Singer separatory flask. Extract with 4 portions of washed ether, using the same procedure as detailed above with a total of about 40 cc. of ether. Evaporate the combined ether extractions in a 50 cc. tared beaker at room temperature, dry over sulfuric acid and weigh. The residue, if it is pure white and has the characteristic odor of vanillin, may be considered vanillin. If the residue is discolored or gummy or has not crystallized after standing in the desiccator overnight, it should be extracted with 15 successive small portions of boiling petroleum ether. The petroleum ether extracts are combined and evaporated in a small, tared beaker and the residue, which should be vanillin, dried over sulfuric acid and weighed. A series of separatory funnels can be used instead of the separatory flask. Vanillin melts at approximately 80° C. Dissolve a small quantity of vanillin in hydrochloric acid. Add a few crystals of resorcinol. A red to pink coloration should be produced.

A more rapid, though somewhat less accurate method of estimating the amount of vanillin is to heat the dry residue at 105° C. for 2 hours, cool, reweigh and regard the loss as vanillin. Prior to heating the vanillin may be sublimed and the sublimate subjected to the characteristic test for vanillin.[9]

Coumarin: Evaporate at room temperature, in a small tared beaker, the original ether extract obtained from the sample from which the vanillin has been removed by means of the ammonia washings. Dry over sulfuric acid and weigh. If the residue is pure white, identification tests may be made on it directly. If not, the residue may be purified by

[9] Hiltner, U. S. Dept. of Agr., Bur. Chem., Bull. No. 152 (1911).

recrystallization from petroleum ether in the manner described under vanillin. Pure coumarin melts at approximately 67° C. and has a characteristic odor resembling "sweet grass" and tonka beans.

The presence of coumarin may be confirmed by applying the Leach[10] test. Dissolve some of the residue in a few drops of water by warming, and transfer to a small porcelain dish or to a spot plate. Add a few drops of 0.1 N iodine solution. Coumarin yields a brown precipitate which gathers into green flecks, leaving a clear brown solution.

Wichmann[11] Rapid Method for Coumarin—If coumarin, alone, is to be determined, transfer 50 cc. of the vanilla extract to a 100 cc. volumetric flask, add lead acetate in slight excess, make up to volume and filter. Remove the excess lead by means of anhydrous potassium oxalate and filter. Extract 50 cc. of the deleaded filtrate with three or four portions of washed ether. Add to the ether layers, a few drops of phenolphthalein indicator and a slight excess of alcoholic potash. Remove the vanillin salt by washing with several 10 cc. portions of water until the disappearance of the pink phenolphthalein color in the wash water shows the absence of alkali. Evaporate the washed ether solution in a small tared dish, dry and weigh the coumarin.

Folin and Denis[12] Colorimetric Method for Vanillin

This method is based on the production of an intense blue color due to the reduction of the complex phosphotungsticmolybdic reagent by vanillin which is a phenolic compound.

Reagent: Phosphotungstic-phosphomolybdic acid—To 100 g. of sodium tungstate and 20 g. of phosphomolybdic acid or its equivalent of molybdic acid, add 100 g. of 85 per cent phosphoric acid and 700 cc. of water. Boil over a free flame for 24 hours, making up the loss by evaporation by the addition of water. Cool, filter, and make up to 1 liter.

Determination. Transfer to a 100 cc. volumetric flask 5 cc. of the vanilla extract or less if the sample contains more than 8–12 mg. of vanillin per 5 cc. Add 75 cc. of water, 4 cc. of lead acetate solution, containing 5 per cent basic lead acetate and 5 per cent neutral lead acetate, make up to volume and mix. Filter rapidly through a dry filter

[10] Leach Winton, "Food Inspection and Analysis," p. 923, Wiley (1920).
[11] Wichmann, *Ind. Eng. Chem.* 10, 537 (1918).
[12] Folin and Denis, *Ind. Eng. Chem.* 4, 670 (1912).

and transfer 5 cc. of the clear filtrate by means of a pipette to a 50 cc. volumetric flask. Into another 50 cc. volumetric flask, pipette 5 cc. of standard vanillin solution (1 cc. = 0.1 mg. of vanillin). To each of these flasks add by means of a pipette 5 cc. of the phosphotungstic-phosphomolybdic reagent, allowing it to flow down the neck of the flask in such a way as to wash down the vanillin solution that may be on the sides of the flask. Mix the contents of the flasks by rotating and, after 5 minutes, dilute the contents to 50 cc. with saturated sodium carbonate solution. Mix thoroughly by inverting the flasks several times and allow to stand for at least 10 minutes, so that the precipitate that forms may separate completely. Filter the solutions through a dry filter and compare the blue colors produced in a colorimeter. Calculate in the usual manner and report as g. of vanillin per 100 cc. of extract.

Wichmann[13] Test for Coumarin

This test is based on the conversion of coumarin to potassium salicylate when it is fused with potassium hydroxide whereas vanillin under the same conditions is converted to potassium protocatechuate.

To 10 cc. of the vanilla extract add 10 per cent sodium hydroxide, sufficient to make the solution alkaline. Dilute with 15 cc. of water to reduce the alcoholic strength and extract with 20 cc. of ether in a separatory funnel. The ether layer will be slightly colored when the brown aqueous layer has been drawn off. Add a few cc. of strong alcoholic potash, shake the mixture and wash with 10 cc. of water. The ether layer should then be colorless. This procedure removes all organic acids, vanillin, coloring matter or saccharin that may be present. Place 1 cc. of 50 per cent potassium hydroxide solution in a test tube. Overlay the potassium hydroxide in the test tube with the ether layer, shake thoroughly and rapidly evaporate the ether on a steam or water bath. Heat the tube over a free flame, evaporating the water. Fuse the potassium hydroxide. If coumarin is present in any amount a change of color will be noticed as the evaporation of the water proceeds and fusion begins. Even very small quantities of coumarin in strong, hot potassium hydroxide solution will show a greenish yellow color that suddenly disappears as the heating is continued. The disappearance of the color shows that the coumarin has been converted into the salicylate and heating should be discontinued. Take up the melt with a few cc. of water, acidify with sulfuric acid and

[13] Wichmann, *Ind. Eng. Chem.* 10, 536 (1918).

extract in a small separatory funnel with 5–10 cc. of benzene. Benzene is preferable as the solvent, because it will dissolve little of the mineral acid and the protocatechuic acid formed from vanillin that may have been carried over with the ether. Remove the acid layer from the funnel, wash the benzene into a test tube, and test for salicylic acid with a cc. of water containing a few drops of ferric chloride solution. If no color develops on shaking, neutralize any trace of mineral acid that may be present and prevent the development of the purple color, by the addition of one or two drops of 0.1 *N* sodium hydroxide.

The change of color on fusion indicates its own end point, and gives, together with the purple salicylate color, a double test for coumarin. Coumarin is changed to the salt of coumaric acid by the hot potassium hydroxide. The development of the yellow color shows this phase. The sudden disappearance of the color indicates the conversion into the colorless salicylate.

Lead Number

When a lead salt is added to vanilla extract, a precipitate is produced, in a similar manner as in maple products, by materials whose lead salts are insoluble. In the case of vanilla extract, however, only neutral lead acetate may be used, for basic lead acetate yields a precipitate with vanillin. The residual lead, that is, the amount of lead not precipitated, is determined by either the Winton [14] or the Wichmann [15] method and is known as the Winton or Wichmann lead number. Since these lead numbers are empirical, the details of the methods must be followed minutely in order to obtain results that have meaning. The directions of the A. O. A. C. follow.

Winton Method—Determine lead as sulfate or chromate in 10 cc. of the filtrate from the lead acetate precipitate of the vanillin and coumarin gravimetric determination, and in the filtrate from a blank determination, using water and 5 drops of glacial acetic acid in place of the sample as directed under the Wichmann number. Calculate the lead number and report as "Lead Number—Winton."

Wichmann Method—Place 175 cc. of boiled water in a round-bottomed flask of 1 liter capacity. Add by means of a pipette 25 cc. of clear

[14] Winton, U. S. Dept. of Agr., Bur. Chem., Bull. No. 95 (1912).
[15] Wichmann, *Ind. Eng. Chem.* 13, 414 (1921).

lead acetate solution, 8 g. per 100 cc., and 50 cc. of sample. Place the flask in a hole in an asbestos board that is large enough to prevent the heating of the upper portion of the flask. When the contents of the flask are reduced to 50 cc. of liquid, the level of the liquid should be even with the top of the board, or slightly above it. Connect the flask to a condenser, and with a moderate flame distill 200 cc. into a volumetric flask, reserving the distillate for the determination of alcohol, if desired. Transfer the residual solution to a 100 cc. volumetric flask by means of carbon dioxide-free water and a bent glass rod provided with a rubber tip. When cool, dilute to 100 cc. with carbon dioxide-free water, mix, and filter through a dry filter (Solution A). Conduct a blank determination, using 5 drops of glacial acetic acid in place of the sample and distilling 150 cc. instead of 200 cc. Determine lead as directed below, either as sulfate or chromate, and calculate the lead number and report as "Lead Number—Wichmann."

Determination of Lead—*As sulfate:* Pipette 10 cc. of Solution A into a 250 cc. beaker, add 25 cc. of water, 2 cc. of sulfuric acid (1 : 1) and 100 cc. of 95 per cent alcohol, stir, and allow to settle overnight. Filter on a Gooch crucible, wash with 95 per cent alcohol, ignite at low redness, cool in a desiccator, and weigh. The difference between the weight of lead sulfate obtained from the blank and that obtained from the sample $\times$ 13.66 = the lead number of the extract.

As chromate: Pipette 10 cc. of solution A into a 400 cc. beaker and add 2 cc. of glacial acetic acid, 25 cc. of water, and 25 cc. of approximately 0.1 *N* potassium dichromate solution. Heat the beaker and contents immediately with a moderate flame until the precipitate changes in color from yellow to orange. Filter on a Gooch crucible; wash thoroughly with hot water and then with a few cc. each of alcohol and ether. Dry at 100° C., cool in a desiccator, and weigh. The difference between the weight of lead chromate obtained from the blank and that obtained from the sample $\times$ 12.82 = the lead number.

VINEGAR

The Definitions and Standards for Food Products of the Food and Drug Administration, U. S. Dept. Agr. (Service and Regulatory Announcements, Food and Drug No. 2, Rev. 5 (1936)) defines vinegars as follows:

1. *Vinegar, Cider Vinegar, Apple Vinegar* is the product made by the alcoholic and subsequent acetous fermentations of the juice of apples. It contains, in 100 cc. (20° C.) not less than 4 g. of acetic acid.

2. *Wine Vinegar, Grape Vinegar* is the product made by the alcoholic and subsequent acetous fermentations of the juice of grapes. It contains, in 100 cc. (20° C.) not less than 4 g. of acetic acid.

3. *Malt Vinegar* is the product made by the alcoholic and subsequent acetous fermentations, without distillation, of an infusion of barley malt or cereals whose starch has been converted by malt. It contains, in 100 cc. (20° C.) not less than 4 g. of acetic acid.

4. *Sugar Vinegar* is the product made by the alcoholic and subsequent acetous fermentations of sugar syrup, molasses, or refiners syrup. It contains, in 100 cc. (20° C.) not less than 4 g. of acetic acid.

5. *Glucose Vinegar* is the product made by the alcoholic and subsequent acetous fermentations of a solution of glucose. It is dextrorotatory and contains, in 100 cc. (20° C.) not less than 4 g. of acetic acid.

6. *Spirit Vinegar, Distilled Vinegar, Grain Vinegar* is the product made by the acetous fermentation of dilute distilled alcohol. It contains in 100 cc. (20° C.), not less than 4 g. of acetic acid.

Because vinegars may be made in a variety of ways, although all by means of acetous fermentation, and furthermore because of the liberality of governmental definitions, it is more or less easy to reconstitute a vinegar to simulate a natural product. The use of vinegar as a condiment is probably as old as the use of wine for it was very likely first obtained from subsequent acetous fermentation of wine. It is well to remember that even though vinegar is essentially a dilute solution of acetic acid, it is a product of fermentation and consequently contains many characteristic substances that are not present in an imitation vinegar. The determination of the presence of these other fermentation products provides an index of its genuineness. In Tables 43, 44, the analyses of a number of typical vinegars are given. As in other food materials, analysis of a vinegar and comparison with the normals will at times indicate whether or not it is standard or substandard. More skillful sophistication may be detected by other means.

In general the analysis of vinegar proceeds along lines that have been previously detailed. Specific gravity, solids, ash, soluble ash, insoluble ash, alkalinity of ash and acidity may be estimated as usual. Fixed acids, which in the case of vinegar is largely lactic acid and not malic acid, volatile acids and alcohol are determined as detailed in the chapter on alcoholic beverages, Chapter XIV. Phosphates and other inorganic de-

terminations may be made as detailed in the chapters on inorganic determinations and metals, Chapters XVII and V. Sugars and reducing substances are estimated as outlined in Chapter IX. Alcohol precipitate, dextrin, pentosans, characteristic organic acids in a manner similar to

TABLE 43. COMPOSITION OF VINEGAR [16,17]

	Average	Maximum	Minimum
Total acid as acetic acid %	4.94	7.96	3.29
Total solids %	2.54	4.52	1.37
Non-sugar solids %	1.90	2.89	1.26
Reducing sugars in solids %	19.6	45.0	5.0
Total ash %	0.367	0.52	0.20
Alkalinity of water soluble ash, cc.	35.7	56.0	21.5
Ash in non-sugar solids %	18.8	26.5	11.2
Soluble phosphoric acid (mg. P_2O_5)	17.3	39.9	6.7
Insoluble phosphoric acid (mg. P_2O_5)	12.0	32.0	4.3
Total phosphoric acid (mg. P_2O_5)	29.3	64.2	15.1
Polarization (direct) V°	—1.46	—3.6	—0.2
Polarization (invert) V°	—1.69	—3.1	±0.0

TABLE 44. CHEMICAL COMPOSITION OF CIDER VINEGARS [18]

	Filtered[19]	Cleared[19]
Sp. gr. $\frac{15°C.}{15°C.}$	1.0193	1.0153
Alcohol% by vol.	0.04	0.055
Glycerol	0.235	0.245
Solids	1.315	1.205
Sugars as invert		
Before Inversion	0.45	0.45
After Inversion	0.12	0.11
Non-sugar solids	1.19	1.10
Volatile reducing substances	0.33	0.34
Total acid as acetic	6.47	6.63
Volatile acid as acetic	6.44	6.58
Fixed acid as malic	0.03	0.055
Volatile esters as ethyl acetate	0.80	0.88
Pentosans	0.083	0.076
Formic acid	0.0004	0.0004
Ash	0.30	0.31
Alkalinity of ash cc. 0.1 *N* acid per 100 cc.		
Soluble ash	34.5	34.6
Insoluble ash	5.8	5.9

16 Balcom, U. S. Dept. Agr., Bull. No. 132 (1909).

17 Of about 100 samples, in g. per 100 cc.

18 Hartman and Tolman, *Ind. Eng. Chem.* 9, 759 (1917).

19 In g. per 100 cc. unless otherwise stated.

the methods given in the chapters on jams and jellies and gums, Chapters XI and X. The methods for glycerol, for the detection of mineral acids and for the differentiation between natural and artificial vinegars will be discussed more fully and particularly.

Glycerol

The official A. O. A. C. method is based on the separation of the glycerol in vinegars from other oxidizable materials, the oxidation of the glycerol by strong potassium dichromate solution and the subsequent calculation of the amount of glycerol from a control oxidation of ferrous ammonium sulfate.

Reagents: (a) Strong potassium dichromate solution. Dissolve 74.55 g. of dry recrystallized potassium dichromate in water; add 150 cc. of sulfuric acid; cool, and dilute with water to 1 liter at 20° C. One cc. of this solution = 0.01 g. of glycerol. Owing to the high coefficient of expansion of this strong solution, it is necessary to make all volumetric measurements of the solution at the same temperature as that at which it was diluted to volume.

(b) Dilute potassium dichromate solution. Measure 25 cc. of the strong potassium dichromate solution at 20° C. into a 500 cc. volumetric flask and dilute to the mark with water at room temperature. Twenty cc. of this solution = 1 cc. of (a).

(c) Ferrous ammonium sulfate solution. Dissolve 30 g. of crystallized ferrous ammonium sulfate, $Fe(NH_4)_2(SO_4)_2.6H_2O$, in water, add 50 cc. of sulfuric acid, cool, and dilute with water to 1 liter at room temperature. 1 cc. of this solution = approximately 1 cc. of (b). As its value changes slightly from day to day, it must be standardized against (b) whenever used.

(d) Diphenylamine indicator. Dissolve 1 g. of diphenylamine in 100 cc. of sulfuric acid.

(e) Retarder. Dilute 150 cc. of syrupy phosphoric acid with 600 cc. of water and 250 cc. of sulfuric acid.

(f) Milk of lime. Introduce 150 g. of calcium oxide selected from clean hard lumps, prepared preferably from marble, into a large porcelain or iron dish; slake with water, cool, and add sufficient water to make 1 liter.

(g) Silver carbonate. Dissolve 0.1 g. of silver sulfate in about 50 cc. of water, add an excess of sodium carbonate solution, allow the precipitate to settle, and wash with water several times by decantation until the

washings are practically neutral. This reagent must be freshly prepared immediately before use.

Determination: Make evaporations on a water bath maintained at a temperature of 85–90° C. The area of the dish exposed to the bath should not be greater in circumference than that covered by the liquid inside.

Evaporate 100 cc. of the vinegar to 5 cc., add 20 cc. of water, and again evaporate to 5 cc. to expel acetic acid. Treat the residue with about 5 g. of 40 mesh sand and 15 cc. of the milk of lime and evaporate almost to dryness, with frequent stirring, avoiding the formation of a dry crust or evaporation to complete dryness. Treat the moist residue with 5 cc. of water; rub into a homogeneous paste; add slowly 45 cc. of absolute alcohol, washing down the sides of the dish to remove adhering paste; and stir thoroughly. Heat the mixture on a water bath, with constant stirring, to incipient boiling; transfer to a suitable vessel; and centrifuge. Decant the clear liquid into a porcelain dish and wash the residue with several small portions of hot alcohol, 90 per cent by volume, by the aid of the centrifuge. If a centrifuge is not available, decant the liquid through a folded filter into a porcelain dish. Wash the residue repeatedly with small portions of hot 90 per cent alcohol, twice by decantation, and then by transferring all the material to the filter. Continue the washing until the filtrate amounts to 150 cc. Evaporate to a syrupy consistency, add 10 cc. of absolute alcohol to dissolve this residue, and transfer to a 50 cc. glass-stoppered cylinder, washing the dish with successive small portions of absolute alcohol until the volume of the solution is 20 cc. Add 3 portions of 10 cc. each of anhydrous ether, shaking thoroughly after each addition. Let stand until clear, pour off through a filter, and wash the cylinder and filter with a mixture of 2 volumes of absolute alcohol and 3 of anhydrous ether. If a heavy precipitate has formed in the cylinder, centrifuge at low speed, decant the clear liquid, and wash 3 times with 20 cc. portions of the alcohol-ether mixture, shaking the mixture thoroughly each time and separating the precipitate by means of the centrifuge. Wash the paper with the alcohol-ether mixture and evaporate the filtrate and washings on the water bath to about 5 cc.; add 20 cc. of water, and again evaporate to 5 cc.; again add 20 cc. of water and evaporate to 5 cc.; finally add 10 cc. of water and evaporate to 5 cc.

These evaporations are necessary to remove all the ether and alcohol, and when conducted at 85–90° C. they result in no loss of glycerol if the concentration of the latter is less than 50 per cent.

Transfer the residue with hot water to a 50 cc. volumetric flask, cool, add the silver carbonate prepared from 0.1 g. of silver sulfate, shake, and

allow to stand 10 minutes. Then add 0.5 cc. of basic lead acetate solution, prepared as directed in the section "Clarifiers" in Chapter IX; shake occasionally, and allow to stand 10 minutes. Make up to the mark, shake well, filter, rejecting the first portion of the filtrate, and pipette 25 cc. of the clear filtrate into a 250 cc. volumetric flask.

Add 1 cc. of sulfuric acid to precipitate the excess of lead and then 30 cc. of reagent (a), the strong potassium dichromate solution. Add carefully 24 cc. sulfuric acid, rotating the flask gently to mix the contents and avoid violent ebullition, and then place in a boiling water bath for exactly 20 minutes. Remove the flask from the bath, dilute, cool, and make up to the mark at room temperature. The quantity of strong dichromate solution used must be sufficient to leave an excess of about 12.5 cc. at the end of the oxidation, the quantity given above, namely 30 cc., being sufficient for ordinary vinegar containing about 0.35 g. or less of glycerol per 100 cc.

Standardize the ferrous ammonium sulfate solution by pipetting 20 cc. into a 250 cc. beaker, adding 20 cc. of the retarder, 4 drops of the indicator, and about 100 cc. of water. Titrate with the dilute potassium dichromate solution until the liquid assumes a dark green color, then add the dichromate slowly dropwise, stirring continuously, until the color changes from a blue gray to a deep violet. Designate the cc. of dilute dichromate solution used as (a). In place of the dilute dichromate solution, substitute a burette containing the oxidized glycerol with an excess of the strong dichromate solution and titrate 20 cc. of the ferrous ammonium sulfate solution as before, designating the cc. used as (b).

From the figures obtained calculate the glycerol, g. per 100 cc. of vinegar by the following formula

$$G = \left[D - \frac{250(a)}{20(b)} \right] 0.02$$

in which,

G = g. of glycerol per 100 cc. of vinegar

and,

D = cc. of the strong potassium dichromate solution used to oxidize the glycerol.

Free Mineral Acids

Many tests used to detect free mineral acids in vinegars depend on the fact that, in general, the hydrogen ion concentration will be much

greater for a certain percentage of mineral acid than for a corresponding percentage of organic acid. This greater hydrogen ion concentration may easily be demonstrated by use of an indicator which will be changed to a different color if the pH falls below a certain value.

Allen's[20] *test, Logwood Method:* Prepare an extract of logwood. Pour 100 cc. of boiling water upon 2 g. of fresh logwood chips, allow the infusion to stand for a few hours, and filter. Place several drops of the liquid on a porcelain surface or spot plate and dry on a water bath. Add to one of the spots a drop of the vinegar to be tested and evaporate to dryness. A yellow tint remains if free mineral acids are absent, and a red tint if they are present.

Methyl Violet Method: Add 5–10 cc. of water to 5 cc. of vinegar and after mixing add 4 or 5 drops of methyl violet solution, 1 part of methyl violet 2B in 10,000 parts of water. A blue or green color indicates the presence of a free mineral acid.

Topfer's Reagent, 0.5 g. dimethylaminobenzene in 100 cc. 95 per cent alcohol may be used in a manner similar to that of the methyl violet method. In the presence of mineral acid the color of the indicator changes from yellow or salmon pink to red.

The A. O. A. C. gives the following details for a tentative method for the quantitative estimation of mineral acids. To a measured quantity of the sample add a measured excess of standard alkali, evaporate to dryness, incinerate, and titrate the ash with standard acid, using methyl orange indicator. The difference between the number of cc. of alkali first added and the number of cc. of acid needed to titrate the ash represents the free mineral acid present.

Detection of Artificial Vinegar

The problem of detecting artificial vinegars and distinguishing between spirit vinegar and other fermentation vinegars is not always easy. The definitions of the Food and Drug Administration are so drawn as to exclude substances not mentioned, and since color is not designated, the presence of color in an unknown sample of vinegar would be indicative of artificiality. However, a far better means of distinction is to determine the presence or absence of characteristic substances normally present in natural vinegars. One of these characteristic substances is acetylmethylcarbinol. This substance is also present in very small amounts in butter.

[20] Allen's "Com'l Organic Analysis," 5th ed., Vol. I, Blakiston (1923).

Methods for the differentiation of fermentation vinegar from artificial vinegar by estimating acetylmethylcarbinol have been developed.

One of these methods is that noted by Dingemans [21] based on the distillation of the acetylmethylcarbinol in the presence of ferric chloride with the formation of biacetyl which may then be estimated as nickel dimethylglyoxime as detailed under the section, biacetyl, in Chapter VII. A more rapid method depends upon the reduction of cold Fehling's solution.[22] The cuprous oxide so formed is transferred to a weighed, narrow centrifuge tube, in which it is washed by centrifuging with water, alcohol and ether, dried at 30° to 40° C., and weighed.

Genuine fermentation vinegar contains only very little formic acid,[23] but artificial vinegar made from acetic acid manufactured from acetylene, even when controlled during the process of manufacture by means of the permanganate test, may contain relatively large amounts of formic acid. This may be estimated as described in the chapter on preservatives, Chapter IV. This determination is of no value as a means of detection when pure commercial acetic acid is used.

There are a number of other methods for the differentiation of, not only fermentation vinegar from artificial vinegar, but also for the differentiation of wine vinegar from spirit vinegar, as well as from artificial vinegar. These methods are based on the oxidation of the reducing substances normally present in fermentation vinegar and absent in artificial vinegar and spirit vinegar. Thus Schmidt [24] defines the "oxidation value" of a vinegar as the number of cc. of 0.1 *N* potassium permanganate solution required to give a permanent pink color to 50 cc. of vinegar containing 3 per cent acetic acid, in the presence of sulfuric acid. If the vinegar contains more than 3 per cent acetic acid, it must be diluted to that value, and if it contains caramel color, it must be decolorized with active carbon by treatment for 2 minutes at room temperature. The oxidation value should be determined before and after treatment with the active carbon.

Determination: [25] Take a quantity of vinegar sufficient to yield a solution of 3 per cent acidity when diluted to 100 cc. To such quantity add 1 g. of activated carbon, dilute to a volume of 100 cc., shake for 2 minutes and filter. Take 50 cc. of the filtrate, add 2 cc. of 1 : 1 sulfuric acid,

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[21] Dingemans, *Ann. fals.* 26, 346 (1933).

[22] Arbenz and Pritzker, *Mitt. Lebensm.* 22, 354 (1931).

[23] Kreutz and Buchner, *Z. Untersuch. Lebensm.* 52, 295 (1926).

[24] Schmidt, *Z. Untersuch. Lebensm.* 73, 441 (1937).

[25] Conn. Agr. Exptl. Sta. Rep., *Analyst* 62, 550 (1937).

heat just to boiling and titrate with 0.1 *N* potassium permanganate, until the pink color developed lasts for half a minute. The volume in cc. of 0.1 *N* potassium permanganate consumed, corrected for a blank determination made with water carried through the same procedure, is the oxygen or oxidation value of the vinegar.

The end-point is fugitive, except with uncolored dilute acetic acid, and cannot be determined with the same degree of accuracy as in inorganic permanganate determinations. For wine, the value is 50 or more; for wine vinegar of acidity 9.2 to 9.4 per cent, the value is about 30, and for wine vinegar of 3 per cent strength, it varies from 8 to 12. Distilled vinegar has values about 5; artificial vinegar, generally not more than 1 to 1.5 and dilute acetic acid has a value of zero. The method cannot be applied easily to cider vinegars because the end-point cannot be read to even approximate accuracy owing to the large amount of manganese dioxide formed. They give oxygen values well over 100.

Another means of determining the amount of oxidizable material is to use iodine solution. The iodine value may be ascertained by adding excess 0.01 *N* iodine solution, measured accurately, to 25 cc. of the vinegar, which has been made alkaline. The excess iodine must be at least 25 per cent. The mixture is allowed to stand for 15 minutes, then is acidified with dilute hydrochloric acid, and the iodine is back-titrated with 0.01 *N* sodium thiosulfate solution. The estimation is made both before and after treatment with active carbon for 2 minutes at room temperature, and samples containing more than 3 per cent of acetic acid are diluted to that concentration. Spirit vinegar gives values from 30 to 60, wine vinegars from 90 to 300, according to strength, and artificial vinegars from 4 to 7. If the value is below 20 the sample may be regarded with suspicion.

A method using Schiff's reagent, prepared as directed under the section, aldehydes, Chapter XIV, is the following and may be applied in the presence of caramel. To 10 cc. of the vinegar, add 1 cc. of phosphoric acid and 1 cc. of 3 per cent potassium permanganate solution and allow the mixture to stand for 10 minutes. Rapid decolorization takes place with wine vinegars; the decolorization is slower with spirit vinegars and is negligible with artificial vinegars. On the further addition of 1 cc. of saturated oxalic acid solution, 1 cc. of 4 *N* sulfuric acid, followed after a short interval by 1 cc. of Schiff's reagent, the rapid formation of an intense red-violet color is characteristic of wine vinegars, a pale violet of spirit vinegars, and the absence of color of artificial vinegars. The

formation, after some time, of a pale brown color is due to the presence of caramel.

Schmidt suggests as minimum values for wine vinegars corresponding with 3 per cent of acetic acid: Oxidation value, 8 cc. of 0.1 *N* potassium permanganate solution per 50 cc. of 3 per cent vinegar solution; iodine value, 90 cc. of 0.01 *N* iodine solution per 25 cc. of 3 per cent vinegar solution.

REFERENCES

Leach-Winton, "Food Inspection and Analysis," Wiley (1920).
Woodman, "Food Analysis," McGraw-Hill (1931).
Cox, "Analysis of Foods," Blakiston (1926).
Brooks, "Critical Studies in the Legal Chemistry of Foods," Chemical Catalog (1927).
Thurston, "Pharmaceutical and Food Analysis," Van Nostrand (1922).
Methods of A. O. A. C. (1935).
Winton, "Structure and Composition of Foods," Wiley (1932) (1935) (1937).

CHAPTER XIII

NON-ALCOHOLIC BEVERAGES AND ALLIED PRODUCTS

Although coffee, tea and other beverages have little food value in themselves, except for that given by added foodstuffs such as milk, cream and sugar, the flavor, stimulation of appetite, relaxation and satisfaction that they give and produce during and at the end of meals, make them valuable food adjuncts. Cacao products, such as chocolate, are concentrated foods and have actual food value as well as flavoring and stimulating properties.

The exhilarating effects of coffee, tea, cocoa and some carbonated beverages are due to purine derivatives such as caffeine, theobromine and xanthine which are closely related to uric acid.

COFFEE

The definitions and standards for food products, U. S. Department of Agriculture defines coffee as follows:

Coffee is the seed of cultivated varieties of *Coffea arabica*, *C. liberica*, and *C. robusta*.

a) Green coffee, raw coffee, unroasted coffee, is coffee freed from all but a small portion of its spermoderm, and conforms in variety and in place of production to the name it bears.

b) Roasted coffee, "coffee" is properly cleaned green coffee which by the action of heat, roasting, has become brown and has developed its characteristic aroma.

The beverage, coffee, is the liquid made from the roasted bean by a number of processes commonly known as percolation, decoction, and infusion.

The most important constituents of raw coffee as can be seen from Table 45 are oil, cellulose, water, and reducing sugar. The process of roasting caramelizes the sugar, reduces moisture content and at the same time develops the characteristic flavors of coffee. Only a small amount of caffeine is lost in the roasting process. Comparative compositions are given in Table 46.

TABLE 45. COMPOSITION OF COFFEE [1]

	Raw Coffee		Roasted Coffee	
	Minimum	Maximum	Minimum	Maximum
Water %	8.0	12.0	0.4	4.0
Caffeine %	0.8	1.8	0.8	1.8
Fat %	11.4	14.2	10.5	16.5
Reducing sugar %	5.8	7.8	0.0	1.1
Cellulose %	16.6	42.3	26.3	51.0
Total nitrogen %	1.1	2.2	1.3	2.7
Ash %	3.5	4.0	4.0	5.0

TABLE 46. COMPOSITION OF COFFEE BEFORE AND AFTER ROASTING [1]

	Raw Coffee	Roasted Coffee
Water %	11.23	1.15
Caffeine %	1.21	1.24
Fat %	12.27	14.48
Sugar %	8.55	0.66
Cellulose %	18.17	10.89
Nitrogenous substances %	12.07	13.98
Other non-nitrogenous matter %	32.58	45.09
Ash %	3.92	4.75

TABLE 46a. COMPOSITION OF COFFEE BEFORE AND AFTER ROASTING [2]

	Raw %	Roasted %
Water	10.73	2.16
Sugar	8.62	0.75
Caffeine	1.07	1.20
Crude fiber	24.00	13.03
Ether extract	11.08	13.75 (petroleum ether cold extract)
Aqueous extract	30.35	12.62
Ash	3.00	4.03
Nitrogenous substances	12.64	2.27 (total nitrogen)
Other nitrogen free extractives	19.30	....
Dextrin	0.86	....
Tannic acid	9.02	

[1] König, "Chemie der menschlichen Nahrungs und Genussmittel," Springer (1889).
[2] Bailey, "Food Products and Their Sources," 3rd ed., Blakiston (1928).

Coffee may be analyzed by methods previously described for moisture, ether extract, protein, crude fiber, reducing sugars, ash, etc. Certain types of adulteration may best be detected microscopically, as for example in the case of chicory. However, it is well to note that chicory is one of the substances which contains inulin, which, on hydrolysis, yields levulose. A high levulose content in a coffee would be indicative of the presence of chicory.

Caffeine

Power-Chesnut[3] Method—In this method caffeine is extracted with alcohol, liberated from its combination in the food material by use of magnesia and subsequently isolated by means of chloroform. Moisten with 95 per cent alcohol 10 g. of the sample, prepared by grinding to 30 mesh. Transfer to a Soxhlet or similar extractor, and extract with 95 per cent alcohol for 8 hours or as much longer as is necessary to complete the extraction. Transfer the alcohol extract with the aid of hot water to a porcelain dish containing 10 g. of heavy magnesium oxide in suspension, in 100 cc. of water. Evaporate slowly on a steam bath with frequent stirring to a dry, powdery mass. Rub the residue with a pestle into a paste with boiling water and transfer with hot water to a smooth filter, cleaning the dish with a rubber policeman. Collect the filtrate in a liter flask marked at 250 cc. and wash with boiling water until the filtrate reaches the mark. Add 20 cc. sulfuric acid (1 : 9) and boil gently for 30 minutes with a funnel in the neck of the flask. Cool, filter through a moistened double paper into a separatory funnel, and wash with small portions of sulfuric acid (1 : 199). Extract with 6 successive 25 cc. portions of chloroform. Wash the combined chloroform extracts in a separatory funnel with 5 cc. of 1 per cent potassium hydroxide solution. Filter the chloroform into an Erlenmeyer flask. Wash the potassium hydroxide solution with two 10 cc. portions of chloroform, adding them to the Erlenmeyer flask. Evaporate or distill on a steam bath to a volume of 10–15 cc., transfer with chloroform to a small tared beaker, evaporate carefully with the aid of air, dry for 30 minutes at 100° C., and weigh. Test the purity of the residue, which is considered to be caffeine, by determining nitrogen by the usual method and multiply by the factor 3.464.

Fendler-Stüber[4] Rapid Method—Transfer 10 g. of the sample ground to pass through a 30 mesh sieve to a glass stoppered bottle. Add

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[3] Power and Chesnut, *J. Am. Chem. Soc.* **41**, 1298 (1919).

[4] Fendler and Stüber, *Z. Nahr. Genussm.* **28**, 9 (1914).

10 cc. of ammonium hydroxide (1 : 2) and 200 g. of chloroform. Place in a shaking machine for 30 minutes. Chill in a refrigerator. Pour the entire contents of the bottle on a 24 cm. fluted filter, covering immediately with a watch glass, catching the filtrate in a tared flask resting in an ice bath. Stopper the flask as soon as the solvent ceases to run in a continuous stream and weigh. Evaporate on a steam bath with the aid of a current of air. Digest the residue with 80 cc. of hot water for 10 minutes on the steam bath, shaking frequently, and allow to cool. Treat the solution with 1 per cent potassium permanganate solution using 20 cc. for roasted coffee and 10 cc. for green coffee and let stand for 15 minutes at room temperature, shaking occasionally. Add 2 cc. of a solution consisting of 100 cc. of 3 per cent hydrogen peroxide, acetanilid-free, and 1 cc. of glacial acetic acid. If the mixture is still red or reddish, add the hydrogen peroxide solution, 1 cc. at a time until the excess potassium permanganate is destroyed. Place the flask on a steam bath for 15 minutes and add 0.5 cc. portions of the hydrogen peroxide until the mixture ceases to become lighter. Cool, and filter by suction through a Gooch crucible, washing with cold water. Transfer the filtrate to a separatory funnel and extract 6 times with 25 cc. portions of chloroform. Evaporate the combined chloroform extracts to a small volume, transfer to a small tared beaker, finish the evaporation, dry at 100° C., generally 30 minutes is sufficient, and weigh the residue as caffeine. The weight of caffeine, multiplied by 2000, divided by the weight of the chloroform aliquot obtained from the first filtration, equals the percentage of caffeine in the 10 g. sample.

TEA

Tea is prepared and cured by recognized methods of manufacture from the tender leaves, leaf buds, and tender internodes of different varieties of *Thea sinensis L.* It conforms in variety and place of production to the name it bears; contains not less than 4 per cent nor more than 7 per cent of ash; and meets the provisions of the act of Congress approved March 2, 1897, as amended, regulating the importation and inspection of tea. The beverage is a water infusion and is also called tea.

Tea may be analyzed by methods similar to those that have been described in other sections of the book. Thus moisture, ash, petroleum ether extract, inorganic materials, protein, crude fiber, and volatile oil may be estimated by the methods detailed under those headings. Facing due to minerals may be detected in the ash. Caffeine may be estimated

by the Power-Chestnut method or by the one subsequently detailed. Tables 47, 48 and 49 give the composition of tea.

Water Extract

To 2 g. of the ground sample in a 500 cc. flask, add 200 cc. of water and boil over a low flame for 1 hour, rotating occasionally. Close the flask with a rubber stopper through which passes a glass tube 30 in. long for a condenser. Boil very slowly so that no steam escapes from the top of the air condenser. Cool, dilute to volume, mix thoroughly, and filter through a dry filter paper. Transfer an aliquot of 50 cc. to a weighed dish and evaporate to dryness on a steam bath. Place in the oven, heat at 100° C. for 1 hour, cool, and weigh.

Table 47. Analyses of Tea [5]

Type	Moisture %		Total ash %	Water soluble ash %	Water Insoluble ash %	Hot Water Extract %
Green tea (26)[6]	max.		8.39	4.01	5.59	40.08
	min.		6.13	2.80	2.25	30.82
	ave.	5.18	7.16	3.61	3.54	37.41
Black tea (53)[6]	max.		7.42	4.75	3.82	44.92
	min.		5.57	2.87	1.94	28.48
	ave.	5.94	6.27	3.58	2.69	36.23
Mixed tea (10)[6]	ave.	6.16	6.72	3.72	3.00	34.35

Table 48. Composition of Green Tea [7]

	Minimum %	Maximum %
Moisture	6.42	9.83
Extract	45.4	56.6
Total ash	5.5	10.85
Water soluble ash	2.3	4.3
Water insoluble ash	1.8	7.75
Ash insoluble in HCl	0.3	3.73
Silica	0.1	2.7
Tannin	3.98	13.35
Caffeine	1.77	3.83

[5] Winton, Ogden and Mitchell, Conn. Agr. Exptl. Sta. Ann. Rep. (1898).

[6] Number of samples.

[7] McLachlan and Stern, *Analyst* **59**, 385 (1934).

TABLE 49. COMPOSITION OF TEA[8]

	Maximum %	Minimum %	Average %
Water	16.20	3.93	9.51
Protein	36.61	15.91	24.50
Caffeine	4.70	1.00	3.58
Tannin	26.13	8.16	15.65
Ash	8.37	3.82	5.65
Essential oil	0.89	0.54	0.68

CAFFEINE

Bailey-Andrew[9] Method—To 5 g. of the sample, prepared by grinding so that it will pass through a 30 mesh sieve, in a 500 cc. volumetric flask, add 10 g. of heavy magnesium oxide and 200 cc. of water. Boil gently over a low flame for 2 hours, using a small bore glass tube 30 in. long as a condenser. Cool, dilute to volume, and filter through a dry paper. Transfer an aliquot portion of 300 cc., equivalent to 3 g. of original material, to a flask of 1 liter capacity; add 10 cc. of sulfuric acid (1 : 9); and boil until the volume is reduced to about 100 cc. Filter into a separatory funnel, washing the flask with small portions of sulfuric acid (1 : 99), and shake 6 times with chloroform, using 25, 20, 15, 10, 10, 10 cc. portions. Treat the combined extracts with 5 cc. of a 1 per cent solution of potassium hydroxide and when the liquids have completely separated draw off the chloroform layer into a suitable flask or beaker. Wash the alkaline solution in the separatory funnel with 2 portions of chloroform of 10 cc. each, and unite the washings with the main bulk of extract. Evaporate or distill off the chloroform to a small volume, transfer to a small weighed beaker with the aid of chloroform, evaporate to dryness, and complete drying in an oven at 100° C. Test the purity of the residue by determining nitrogen and multiplying by the factor 3.464. This gives the value for anhydrous caffeine.

TANNIN

Tannin may be estimated by a method similar to that detailed under the section, "Tannin and Coloring Matters" in Chapter XIV. The method

[8] König, "Chemie der menschlichen Nahrungs und Genussmittel," p. 1011, 3rd ed., Springer (1889).

[9] Andrew, *J. Assoc. Official Agr. Chem.* 6, 107 (1922).

estimates tannin by determining its oxidizability by potassium permanganate.

Lowenthal-Procter Method [10,11]—*Reagents:* The reagents are prepared in the same manner as in the aforementioned method and in addition prepare:

Gelatin solution. Soak 25 g. of gelatin for an hour in saturated sodium chloride solution, heat until the gelatin is dissolved, cool, and dilute with a saturated sodium chloride solution to 1 liter.

Acid sodium chloride solution. Acidify 975 cc. of saturated sodium chloride solution with 25 cc. of sulfuric acid.

Determination. Boil 5 g. of the tea for 30 minutes with 400 cc. of water, cool, transfer to a 500 cc. volumetric flask, and dilute to the mark. To 10 cc. of the infusion, filtered, if not clear, add 25 cc. of the indigo carmine solution and about 750 cc. of water. Add the potassium permanganate solution from a burette, a little at a time while stirring, until the color becomes light green, then dropwise until the color changes to bright yellow or to a faint pink at the rim. Designate the number of cc. of potassium permanganate used as a.

Mix 100 cc. of the clear infusion of tea with 50 cc. of the gelatin solution, 100 cc. of the acid sodium chloride solution, and 10 g. of powdered kaolin, and shake several minutes in a stoppered bottle. After allowing the mixture to settle, decant through a filter. Mix 25 cc. of the filtrate with 25 cc. of the indigo carmine solution and about 750 cc. of water and titrate with potassium permanganate solution as before. The number of cc. of potassium permanganate solution used subtracted from that obtained above, a, gives the quantity of potassium permanganate solution required to oxidize the tannin. One cc. of 0.1 N oxalic acid equals approximately 0.0042 g. of tannin (gallotannic acid).[12]

CACAO PRODUCTS

The analysis of cacao or cocoa products follow methods described in the foregoing text. Thus moisture, ash, soluble and insoluble ash, alkalinity of ash and protein content may be estimated as previously detailed. Crude fiber in cocoa products not containing milk products is determined on the dried residue after exhaustively washing by centrifuging and

10 Lowenthal, *Z. anal. Chem.* **16**, 33 (1877).

11 Procter, *Chem. News* **37**, 256 (1878).

12 Mitchell, *Analyst* **61**, 295 (1936).

decanting successive portions of ether, water and then, alcohol, and water. Crude fiber in cocoa products containing milk products is determined on the dried residue after exhaustively washing by centrifuging and decanting successive portions of ether, 1 per cent sodium oxalate solution, in which solution the sample is allowed to steep for 30 minutes, water, and then, alcohol, and water. The fat in cocoa products may be extracted by use of ether and recovered by evaporation of the ether. The fat may then be subjected to the examination detailed in Chapter VIII. The qualitative presence of lactose may be established by defatting the cocoa product and then proceding along lines given in Chapter VII. Quantitatively the sucrose and lactose content is ascertained by a combination of the polariscopic and copper reducing methods outlined in Chapter IX, with special formulae and corrections for the sucrose content in the lactose determinations.

The Federal Government definitions for these products follow and the composition of some cocoa products are given in Table 50.

TABLE 50. ANALYSES OF COCOA PRODUCTS [13]

	Plain chocolate	Sweet chocolate	Cocoa	Cocoa shells
Moisture %	3.78	2.17	6.23	4.87
Ash %	3.15	1.40	5.49	10.43
Theobromine %	0.78	0.35	1.15	0.49
Caffeine %	0.13	0.08	0.16	0.16
Protein %	12.36	4.58	18.34	14.46
Crude fiber %	2.86	0.95	4.48	16.55
Sugar %	0.00	56.44	0.00	0.00
Starch %	8.11	2.88	11.14	4.13
Other nitrogen-free material %	16.64	7.64	26.32	46.15
Fat %	52.19	23.51	26.69	2.76

DEFINITIONS OF CACAO PRODUCTS

The following definitions are given by the Food and Drug Administration:

1. Cacao beans, cocoa beans are the seeds of trees belonging to the genus *Theobroma*, especially those of *T. cacao* L. and closely related species.

[13] Winton, Bailey and Silverman, Conn. Agr. Exptl. Sta. Ann. Rep. (1903).

2. Cacao nibs, cocoa nibs, "Cracked cocoa" are roasted or dried cacao beans, broken and freed from germ and from shell or husk.

3. Cacao butter, cocoa butter is the edible fat obtained from sound cocoa beans either before or after roasting.

4. Chocolate, plain chocolate, bitter chocolate, chocolate liquor, chocolate paste, bitter chocolate coating is the solid or plastic mass obtained by grinding cocoa nibs. It contains not less than 50 per cent cocoa fat and, on the moisture and fat-free basis, not more than 8 per cent of total ash, not more than 0.4 per cent ash insoluble in hydrochloric acid, nor more than 7 per cent crude fiber.

5. Sweet chocolate, sweet chocolate coating is chocolate mixed with sugar and/or dextrose, with or without the addition of cocoa butter, spices, or other flavoring materials. It contains on the moisture-, sugar-, and fat-free basis, no greater percentage of total ash, ash insoluble in hydrochloric acid, or crude fiber, respectively, than is found in moisture- and fat-free chocolate.

6. Milk chocolate, sweet milk chocolate is the product obtained by grinding chocolate with sugar and/or dextrose, with the solids of whole milk, or the constituents of milk solids in proportions normal for whole milk, and with or without cocoa butter, and/or flavoring material. It contains not less than 12 per cent milk solids.

7. Cocoa, powdered cocoa is chocolate deprived of a portion of its fat and pulverized. It contains, on the moisture- and fat-free basis, no greater percentage of total ash, ash insoluble in hydrochloric acid or crude fiber, respectively, than is found in moisture- and fat-free chocolate.

8. "Breakfast cocoa" is cocoa which contains not less than 22 per cent cocoa fat.

9. Sweet cocoa, sweetened cocoa is cocoa mixed with sugar and/or dextrose. It contains not more than 65 per cent of total sugars in the finished product, and, on the moisture-, sugar-, and fat-free basis, no greater percentage of total ash, ash insoluble in hydrochloric acid, or crude fiber, respectively, than is found in moisture- and fat-free chocolate.

10. Sweet milk cocoa is the product obtained by grinding cocoa with sugar and/or dextrose, with the solids of whole milk, or the constituents of milk solids in proportions normal for whole milk, and with or without flavoring material. It contains not less than 12 per cent of milk solids.

11. Dutch-Process chocolate, "Alkalized Chocolate," and Dutch-Process cocoa, "Alkalized cocoa" are modifications, respectively of chocolate and cocoa, in that in their manufacture an alkali carbonate, or other

suitable alkaline substance has been employed. In the preparation of these products not more than 3 parts by weight of potassium carbonate, or the neutralizing equivalent thereof in other alkaline substances, are added to each 100 parts by weight of cocoa nibs. The finished products conform to the standards for chocolate and cocoa, respectively, due allowance being made for the kind and amount of alkaline substance added.

Fat

Weigh accurately about 1 g. of chocolate, ground to pass a 30 mesh sieve, or cocoa powder into the bottom section of a Jacobs-Singer separatory flask. Add 10 cc. of water and stir by shaking into a paste. Add 2 cc. of ammonia and again shake vigorously. Boil gently for 5 minutes or else immerse in a boiling water bath for 5 minutes.[14] Cool, stopper with the upper section of the flask. Add 11 cc. of alcohol and proceed with the Roese-Gottlieb method as detailed in Chapter VI, making certain to add additional alcohol before each succeeding extraction with the mixed ethers.

The chocolate may be weighed into a 100 cc. tall form beaker, processed as detailed above and then transferred to a Mojonnier flask or similar apparatus as described in the section "cheese," Chapter VII.

Refractive Method for Total Fat in Cacao Products[15]

Tricresyl phosphate, diethylphthalate, and dibutylphthalate may be used satisfactorily as solvents for a rapid refractometric method for determining total fat in cacao products. The tricresyl phosphate is used for the wide range refractometer, that is, an Abbé refractometer, and the phthalates for the narrow range butyro-refractometer. When equal weights of sample and solvent are taken and the refractive index of the filtrate obtained, the percentage of fat may be read directly from Table 51.

Weigh 20 g. of sample accurately to 0.05 g. and add 20 g. of tricresyl phosphate, also accurately weighed to 0.05 g. and melt in an oven at 100° C. Mix thoroughly. Filter through a 15 cm. No. 1 Whatman fluted filter, by placing the paper in a 100 cc. beaker without using a funnel. Preferably centrifuge in order to obtain the ½ cc. Let the filtration take place in the oven. When about ½ cc. has filtered, read the refractive

[14] Wiseman, *Analyst* **55**, 685 (1930).

[15] Stanley, *Ind. Eng. Chem., Anal. Ed.* **9**, 132 (1937).

index between 35° C. and 45° C., noting the exact temperature. Obtain the percentage fat from Table 51.

TABLE 51. REFRACTOMETRIC DETERMINATION OF FAT IN CHOCOLATE

Cocoa butter per 10 g. of solvent	Refraction 35°C.	40°C.	45°C.
0.000	1.55039	1.54841	1.54659
0.250	1.54772	1.54563	1.54380
0.500	1.54498	1.54283	1.54115
0.750	1.54243	1.54031	1.53823
1.000	1.53892	1.53774	1.53593
1.250	1.53765	1.53533	1.53343
1.500	1.53547	1.53336	1.53167
1.750	1.53348	1.53150	1.52940
2.000	1.53146	1.52950	1.52700
2.250	1.52943	1.52738	1.52548
2.500	1.52774	1.52560	1.52400
2.750	1.52613	1.52445	1.52213
3.000	1.52434	1.52223	1.52049
3.250	1.52283	1.52076	1.51900
3.500	1.52148	1.51953	1.51778
3.750	1.52026	1.51814	1.51649
4.000	1.51856	1.51695	1.51524
4.250	1.51740	1.51540	1.51380
4.500	1.51649	1.51444	1.51253
4.750	1.51515	1.51307	1.51130
5.000	1.51358	1.51177	1.50997
5.250	1.51261	1.51081	1.50921
5.500	1.51185	1.50960	1.50819
5.750	1.51049	1.50906	1.50723
6.000	1.50967	1.50775	1.50619
6.500	1.50825	1.50615	1.50448
7.500	1.50495	1.50309	1.50110
8.500	1.50196	1.50014	1.49828
9.500	1.49950	1.49772	1.49570
10.000	1.49804	1.49627	1.49450

The concentration of cocoa butter is expressed as g. of fat per 10.000 g. of solvent.

The zero point for the solvent is obtained by making a blank test without the fat, and the difference between this figure and that given in the Table 51 is the correction to be used. It seldom exceeds a few units in the fourth decimal place.

In the case of samples running over 60 per cent fat, it is advisable

to double the amount of solvent, and then to double the per cent read in the table. It is preferable to use tricresyl phosphate as the solvent and the wide range refractometer, in order to obtain more accurate results, because of the greater difference in index of refraction between this solvent and cocoa butter.

Butter fat and lecithin do not interfere but coconut oil does.

Milk Proteins

Weigh exactly 10 g. of the finely grated chocolate into a suitable 8 oz. centrifuge bottle. Add two 100 cc. portions of ether, centrifuge and decant the supernatant liquor after each addition. Dry the residue in an oven at about 100° C. and powder the residue in the bottle with a flattened glass rod. Add 200 cc. of 3 per cent sodium oxalate solution and let stand 4 hours, shaking frequently. Centrifuge and filter through a small folded filter. Discard the first 5–10 cc. of the filtrate and determine nitrogen in 50 cc. of this filtrate. Pipette 100 cc. of the filtrate into a 200 cc. volumetric flask and dilute almost to the mark with water. Precipitate the proteins by the addition of 2 cc. of glacial acetic acid. Make to volume, shake, filter, and determine nitrogen in 100 cc. of the filtrate. The difference between the two nitrogen figures obtained is the nitrogen of the casein contained in 2.5 g. of the sample. This figure $\times$ 4 $\times$ 6.38 = the total casein contained in the 10 g. taken for the analysis. Casein $\times$ 1.25 = total milk protein.

Estimation of Butter Fat and Milk Product Content

The quantity of butter fat in cocoa products may be estimated by the following formula based on the assumption of a Reichert-Meissl value of 0.5 for cocoa butter.

$$M.F. = \frac{5R - 2.5}{23.5}$$

in which,

$M.F.$ = g. of butter fat in 5 g. of mixed fat

and

R = Reichert-Meissl value of the extracted fat.

Using the above formula for the calculation of butter fat content and

the foregoing method for the estimation of milk protein, the approximate total milk solids content may be calculated from the following formula :[16]

$$T.M.S. = M.F. + L + M.P. + 0.05\ (M.F. + L + M.P.) \text{ or more simply}$$
$$= 1.05\ [M.F. + L + M.P.]$$

in which,

$T.M.S.$ = per cent total milk solids
$M.F.$ = per cent butter fat
L = per cent lactose
$M.P.$ = per cent milk protein

The lactose content may be approximately calculated from the milk protein content by multiplying the milk protein content by the factor, 1.4. This is an average factor and actually varies about 10 per cent either way but serves for rapid and approximate calculation. The factor 0.05 represents the material to be added to account for milk ash. Janssen and Dehut [17] use the factor 0.0674.

Total Alkaloids [18]

The term alkaloids, as far as cocoa is concerned, means theobromine and caffeine. The cocoa is digested repeatedly with 80 per cent alcohol by volume and a little magnesia and filtered. The filtrate is evaporated, and water is added to replace the alcohol lost. The mixture is clarified with zinc ferrocyanide and again filtered. The filtrate is evaporated to a small volume and extracted with chloroform. Nitrogen is determined in the extract and the total alkaloids are calculated by use of an appropriate factor.

Reagents: Zinc acetate solution. Dissolve 21.9 g. of crystallized zinc acetate, $Zn(C_2H_3O_2)_2.2H_2O$, and 3 cc. of glacial acetic acid in water and make up to 100 cc.

Potassium ferrocyanide solution. Dissolve 10.6 g. of crystallized potassium ferrocyanide in water and make up to 100 cc.

Determination: Grind 2 g. of prepared cocoa or 2 or more g. of cocoa-nib or chocolate to a smooth paste with a little 80 per cent alcohol, and transfer to a 200 cc. flask with more alcohol of the same strength, sufficient to produce a total volume of about 100 cc. Add 1 g. of freshly-ignited

16 Offutt, *J. Assoc. Official Agr. Chem.* 18, 424 (1935).
17 Janssen and Dehut, *Analyst* 61, 45 (1936).
18 Moir and Hinks, *Analyst* 60, 439 (1935).

magnesium oxide and digest in a boiling water bath for $1\frac{1}{2}$ hours under a reflux air condenser, with occasional shaking. Filter while hot through a small Büchner funnel, return the residue to the flask, and re-digest for half an hour with 50 cc. of the alcohol. Filter and repeat the digestion with a further 50 cc. portion of 80 per cent alcohol. Evaporate the combined extract on the water bath, adding hot water from time to time to replace the alcohol lost. When all the alcohol is removed, finally evaporate to about 100 cc., add 2 to 3 drops of 10 per cent hydrochloric acid and transfer the liquid to a 150 cc. volumetric flask. Cool, add 5 cc. of the zinc acetate solution and mix, and then add 5 cc. of the potassium ferrocyanide solution. Make up to the mark with water and mix thoroughly by shaking. Allow the flask to stand for a few minutes, and filter through a dry filter paper. Discard the first portion.

Evaporate a measured quantity, 120 cc., of the filtrate to about 10 cc., transfer to a separatory funnel and extract by vigorous shaking with five successive 30 cc. portions of chloroform. Run off the chloroform after each extraction into a second separatory funnel, and wash the combined extracts with 3 to 5 cc. of water. Repeat the above process of extraction with five successive quantities of chloroform, wash the second chloroform extracts with the same wash water, combine the whole of the extracts, and remove the chloroform by distillation. Dissolve the residue in a little hot water, transfer to a Kjeldahl flask, add 0.2 g. of sucrose and 10 cc. of sulfuric acid. Heat over a small flame until frothing ceases, add 0.02 g. of selenium and digest till colorless. Heat for one hour longer, and determine the ammonia in the usual way by distillation into 0.1 *N* acid. Use the factor 3.26 for the conversion of nitrogen into alkaloid.

CARBONATED BEVERAGES

There are many types of carbonated beverages sold commercially under the name of soda water in the United States. The name has been shortened by usage to "soda", "soda pop" or "pop". The Food and Drug Administration has promulgated definitions and standards for some of these. They are:

Ginger ale is the carbonated beverage prepared from ginger ale flavor, harmless organic acid, water, and a syrup of one or more of the following: sugar, invert sugar, dextrose; with or without the addition of caramel color. Ginger ale flavor or ginger ale concentrate, is the beverage flavor in which ginger is the essential constituent, with or without aromatic and pungent ingredients, citrus oils, fruit juices and caramel color.

Sarsaparilla is the carbonated beverage prepared from sarsaparilla flavor, water, and a syrup of one or more of the following: sugar, invert sugar, dextrose; with or without harmless organic acid, and with or without the addition of caramel color. Sarsaparilla flavor is the beverage flavor prepared from oil of sassafras and methyl salicylate or oil of wintergreen or oil of sweet birch, with or without other aromatic and flavoring substances and caramel color. It derives its characteristic flavor from oil of sassafras and methyl salicylate.

Root beer is the carbonated beverage prepared from root beer flavor, water and a syrup of one or more of the following: sugar, invert sugar, dextrose; with or without harmless organic acid, and with or without the addition of caramel color. Root beer flavor or root beer concentrate, is the beverage flavor in which oil of sassafras and methyl salicylate, or oil of wintergreen or oil of sweet birch, are the principal flavoring constituents. It contains other flavoring substances, with or without the addition of caramel color.

Birch beer is the carbonated beverage prepared from birch beer flavor, water, and a syrup of one or more of the following: sugar, invert sugar, dextrose; with or without harmless organic acid, and with or without the addition of caramel color. Birch beer flavor or birch beer concentrate is the beverage flavor in which methyl salicylate, or oil of sweet birch or oil of wintergreen, and oil of sassafras are the principal flavoring constituents, with or without other flavoring substances, and with or without the addition of caramel color. The flavor of methyl salicylate predominates.

Cream soda water, "cream soda," is the carbonated beverage prepared from cream soda water flavor, water, and a syrup of one or more of the following: sugar, invert sugar, dextrose; with or without harmless organic acid, and with or without the addition of caramel color. Cream soda water flavor or cream soda water concentrate is the beverage flavor prepared from vanilla, tonka, vanillin, or coumarin, singly or in combination, together with other flavoring substances; with or without the addition of caramel color.

No definitions have been promulgated for the so-called fruit beverages both carbonated and not carbonated. Most of the carbonated fruit beverages are in reality imitation fruit beverages, containing little or no fruit extract and consisting in the main of artificial fruit flavor, artificial color, organic and at times mineral acid such as phosphoric acid, sugar, invert sugar or dextrose and carbonated water. Other types of car-

bonated beverages, such as the "cola" type contain added caffeine and act as mild stimulants.

Carbonated beverages may be analyzed by the methods described in other sections of the book. Thus sugars may be estimated as detailed in Chapter IX. The estimation of some organic acids and the presence of mineral acid is outlined in the chapters on jams and jellies and on spices and flavors, Chapters XI and XII. Saccharin and preservatives may be detected as described in Chapter IV. Artificial coloring matters may be detected as detailed in Chapter III.

A method for the estimation of orange juice content of orange drinks is explained in some detail in the chapter on vitamins, Chapter XVI. A procedure for the detection of malted milk powder in malted milk drinks is discussed in the chapter on milk products, Chapter VII.

REFERENCES

Methods of A. O. A. C. (1935).
Woodman, "Food Analysis," McGraw-Hill (1931).
Leach-Winton, "Food Inspection and Analysis," Wiley (1920).
Bailey, "Food Products and Their Sources," 3rd ed., Blakiston (1928).

CHAPTER XIV

ALCOHOLIC BEVERAGES

The province of the food analyst extends to alcoholic beverages not only because they may at times be adulterated but also because of the many federal, State and municipal regulations governing the manufacture, transportation and sale of these products. An indication of what to analyze for, is best obtained from a consideration of the definitions of the various products that fall into this group. Alcoholic beverages may be conveniently divided into three large groups and one parallel group, namely, distilled liquors, wines, beers or malt beverages, and the parallel group cider and vinegar, which although not a beverage is a fermentation product and may be included in the classification.

DISTILLED LIQUORS

A fair idea of the members of the class of distilled liquors may be obtained from the directions given to storekeeper-gaugers in the Gauging Manual of the U. S. Treasury Department on the marking and branding of casks or packages of distilled spirits other than alcohol on entry into distillery warehouses.

Whisky or Whiskey—All spirits (other than alcohol) produced from grain below 160° proof shall be branded "Whiskey".

Such spirits shall be further branded to show the particular kind of whiskey, according to the grain used. Where corn is the principal material used, the spirits will be branded "Bourbon (or Corn) Whiskey" and where rye is the principal material used, the spirits will be branded "Rye Whiskey". If all the grain, or the principal part thereof, is malted, the word "malt" or "malted" shall also be used, as "Rye Malt Whiskey", "Bourbon (or Corn) Malt Whiskey," etc. Other truthful descriptive words may be used such as "straight" or "pure", where whiskey is made in whole from one kind of grain, as "Pure Rye Whiskey," "Pure Rye Malt Whiskey," etc. Legends, if truthful, such as "Sour Mash," "Hand Process," "Copper Distilled," etc. may also be used;

provided, however, that the use of not more than 20 per cent barley malt shall not preclude the spirits being branded "straight" or "pure".

Brandy—All spirits (other than alcohol) produced from fruits shall be branded "Brandy", provided, however, if the spirits are produced from grapes or grape wine and warehoused at or above 160° proof, they may be branded "Fruit Spirits" or "Wine Spirits".

Such spirits shall be further branded to show the particular kind of brandy according to the fruit used, as "Grape Brandy", "Peach Brandy", "Apple Brandy".

Rum—All spirits (other than alcohol) produced from molasses, which by reason of the method of manufacture employed and the characteristic congeneric constituents of the spirits, are known to the trade as rum,[1] shall be branded "Rum".

Gin—All spirits distilled over, or with juniper berries, and having the characteristic constituents of spirits known to the trade as gin, shall be branded "Gin".

Trade Terms

The words, "straight", "blended", "imitation", and "age" are often used in describing whiskies. These terms as indicated above have fixed meanings.

Straight whiskey is the liquor resulting from the distillation of a fermented infusion of grain, the process being carried out in a pot-still, or some other form of still, constructed so that the resulting liquor contains not only the alcohol, but also the greater part of the congeneric substances which are vaporized with the alcohol.

According to the U. S. Pharmacopoeia XI Decennial Revision, whiskey or spiritus frumenti is an alcoholic liquid obtained by the distillation of the fermented mash of wholly or partly malted cereal grains, and containing, at 15.56° C. not less than 47 per cent and not more than 53 per cent by volume of ethyl alcohol. It must have been stored in charred wood containers for a period of not less than four years. It should be a light to deep amber-colored liquid, having a characteristic odor and taste, and an acid reaction. The specific gravity should be 0.935 to 0.923 at 25° C.

[1] Valaer, "Composition of Rum," *Ind. Eng. Chem.* 29, 988 (1937).

Blended whiskey means a mixture of two or more straight whiskies or a mixture of straight whiskey with diluted ethyl alcohol with or without added color or flavor. The blend of straight whiskey and diluted ethyl alcohol is sometimes known as "rectified whiskey".

Imitation whiskey is ethyl alcohol colored and/or flavored in imitation of straight whiskey and which is neither a straight nor a blended whiskey.

Age means the period or time straight whiskey has been stored in wood and for such purposes the words "age" and "old" are synonymous.

Whiskies after storage are sometimes sold as "bottled in bond". This term designates a guaranty that the Federal Government is satisfied that the whiskey is at least 4 years old, that it is standard 100° proof and that nothing has been added since it was distilled except the water necessary to reduce the whiskey to the required proof, and the esters and other materials that the liquor may have acquired during the storage period.

Other terms used in connection with whiskey are the designations, Irish, Scotch, cognac, liqueurs and cordials. Irish whiskey, is a distilled liquor made by a pot-still method originated in Ireland. The peculiar smoky flavor of Scotch whiskey is due to the method of preparing the malt by drying it in kilns over peat fires. Cognac is a fine brandy or wine spirits distilled from grapes, originally made in Charente, France. Now the term is loosely applied to French and foreign brandies in general.

Cordials and liqueurs are prepared by mixing alcohol, water and sugar with various flavoring essences, hence are, in a certain sense of the word, not distilled liquors.

Wine and Proof Gallons

The Gauging Manual cited above defines wine and proof gallons. A wine-gallon of any proof spirits is a standard U. S. gallon containing 231 cu. in.

The proof of spirits is obtained by multiplying the percentage of alcohol by volume by two; therefore, a wine-gallon containing 50 per cent alcohol by volume and 50 per cent water by volume would be 100 proof spirits, and a wine-gallon of 100 proof spirits is known as a proof-gallon.

A wine-gallon of 190 proof spirits would be a standard U. S. gallon containing 231 cu. in. of a mixture containing 95 per cent alcohol by volume and 5 per cent water by volume. If a wine-gallon of 190 proof spirits is diluted with water so that the proof would be 100, the resulting

mixture would be 1.9 standard U. S. gallons of 100 proof spirits; therefore, a wine-gallon of 190 proof spirits is equivalent to 1.9 gallons of 100 proof spirits.

WINES

Wines are generally classified as dry wines or sweet wines with further subdivisions into fortified wines, sparkling, still, etc.

The Service and Regulatory Announcements, Food and Drug No. 2 (Fifth Revision, 1936) U. S. Dept. of Agr., Food and Drug Administration contains the following definitions.

Wine: The product made by the normal alcoholic fermentation of the juice of sound, ripe grapes, and the usual cellar treatment. It contains not less than 7 per cent nor more than 16 per cent of alcohol by volume, and, in 100 cc. (20° C.) not more than 0.1 g. of sodium chloride nor more than 0.2 g. of potassium sulfate; and for red wine not more than 0.14 g., and for white wine not more than 0.12 g. of volatile acids produced by fermentation and calculated as acetic acid.

a) Red wine is wine containing the red coloring matter of the skins of grapes.

b) White wine is wine made from white grapes or the expressed fresh juice of other grapes.

Dry Wine: Wine in which the fermentation of the sugars is practically complete, and which contains, in 100 cc. (20° C.), less than 1 g. of sugars, and for dry red wine not less than 0.16 g. of grape ash and not less than 1.6 g. of sugar-free grape solids and for dry white wine not less than 0.13 g. of grape ash and not less than 1.4 g. of sugar-free grape solids.

Fortified dry wine: Dry wine to which brandy has been added but which conforms in all other particulars to the standard of dry wine.

Sweet Wine: Wine in which the alcoholic fermentation has been arrested and which contains, in 100 cc. (20° C.), not less than 1 g. of sugars, and for sweet red wine not less than 0.16 g. of grape ash, and for sweet white wine not less than 0.13 g. of grape ash.

Fortified Sweet Wine: Sweet wine to which wine spirits have been added. By act of Congress, "sweet wine" used for making fortified sweet wine and "wine spirits" used for such fortifications are defined as follows: "That the wine spirits mentioned is the product resulting from the distillation of fermented grape juice, to which water may have been added prior to, during, or after fermentation, for the sole purpose of facilitating the fermentation and economical distillation thereof, and shall be held to include the product from grapes or their residues com-

monly known as grape brandy, and shall include commercial grape brandy which may have been colored with burnt sugar or caramel; and the pure sweet wine which may be fortified with wine spirits under the provisions of this act is fermented or partially fermented grape juice only, with the usual cellar treatment, and shall contain no other substance whatever introduced before, at the time of, or after fermentation, except as herein expressly provided: Provided, That the addition of pure boiled or condensed grape must or pure crystallized cane or beet sugar, or pure dextrose sugar containing respectively, not less than 95 per cent of actual sugar, calculated on a dry basis, or water, or any or all of them, to the pure grape juice before fermentation, or to the fermented product of such grape juice, or to both, prior to the fortification herein provided for, either for the purpose of perfecting sweet wines according to commercial standards or for mechanical purposes, shall not be excluded by the definition of pure sweet wine aforesaid; Provided, however, That the cane or beet sugar or pure dextrose sugar added for sweetening purposes shall not be in excess of 11 per cent of the weight of the wine to be fortified: And provided, further, That the addition of water herein authorized shall be under such regulations as the Commissioner of Internal Revenue, with the approval of the Secretary of the Treasury, may from time to time prescribe: Provided, however, That records kept in accordance with such regulations as to the percentage of saccharine, acid, alcoholic, and added water content of the wine offered for fortification shall be open to inspection by any official of the Department of Agriculture thereto duly authorized by the Secretary of Agriculture; but in no case shall such wines to which water has been added be eligible for fortification under the provisions of this act, where the same, after fermentation and before fortification, have an alcoholic strength of less than 5 per cent of their volume."

Sparkling Wine: Wine in which the after part of the fermentation is completed in the bottle, the sediment being disgorged and its place supplied by wine or sugar liquor and/or dextrose liquor, and which contains, in 100 cc. (20° C.), not less than 0.12 g. of grape ash.

Modified Wine, Ameliorated Wine, Corrected Wine: The product made by the alcoholic fermentation, with the usual cellar treatment, of a mixture of the juice of sound, ripe grapes with sugar and/or dextrose, or a syrup containing not less than 65 per cent of these sugars, and in quantity not more than enough to raise the alcoholic strength after fermention to 11 per cent by volume.

Raisin Wine: The product made by the alcoholic fermentation of an

infusion of dried or evaporated grapes, or of a mixture of such infusion or of raisins with grape juice.

Wine Types

The more important wines are champagne, port, sherry, claret, burgundy, hock (Rhine or Moselle wine), madeira, marsala, chianti, sauterne, malaga and tokay. Champagne is a sparkling wine produced in Champagne, Loire and Marne in France, Australia, the United States and other places. Port is a strongly fortified red wine originally coming from Portugal. Sherry is another strongly fortified wine coming from Spain. Claret is a light red wine produced in Medoc and the Bordeaux district. It is also made in Hungary. Burgundy is a dark red natural wine native to the eastern part of France but also produced in California. Hock, as mentioned above is a wine manufactured in the vineyards near the Rhine, Moselle and the Main. Madeira is a sherry type of fortified wine. Malaga is another sweet Spanish wine. Chianti is an Italian red wine. Sauterne is a white wine of France. Marsala is a sherry type of wine of Sicilian origin and tokay is, of course, a Hungarian wine.

It is well to remember that many of these wines are produced far from their point of origin and hence have varying characteristics. They are at times the equal of the originals.

BEER, MALT BEVERAGES

Beer is the fermented liquor prepared from malted grain without distillation with or without the addition of hops. It generally contains from 3 to 5 per cent alcohol and is generally brewed from barley grain. There are many types of beer differing mainly in taste qualities due to the addition of different amount of hops and other natural flavoring principles. Lager beer is a German type of beer that is stored several months before use. Bock beer is a beer brewed in the winter for use in the early spring. It contains more alcohol and extract. *Ale* is a beer that is brewed by a variation known as top-fermentation. It usually contains less hops than lager beer and therefore is sweeter. *Porter* is a dark colored malt liquor differing from ale in that it is made from high-dried malt. *Stout* is a porter of higher alcoholic content.

Cider

Cider is the expressed juice of apples, used as a beverage. Before fermentation it is known as sweet cider and after fermentation it is known

as hard cider. Good fermentation produces a product that contains as much as from 3 to 6 per cent alcohol by volume.

Completely and Specially Denatured Alcohol

During the period, 1920-33 known as "Prohibition," it became necessary for the Federal Government to arrange some means of permitting manufacturers and others to obtain alcohol that was needful for the production of numerous articles. In some cases it was virtually impossible to produce a particular item without the use of alcohol. Consequently the Federal Government extended the method of completely or specially denaturing alcohol after which such alcohol could be used for designated purposes. These regulations are published by the Treasury Department.

For complete denaturing, wood alcohol and benzine are used. For special denaturing many substances are used among which may be mentioned: wood alcohol, benzol, commercially pure methyl alcohol, ethyl ether, acetone, ethyl acetate, isopropyl alcohol, diethylphthalate, normal butyl alcohol, refined shellac, phenol, many alkaloids, inorganic materials such as cadmium, potassium and mercuric iodides and many essential oils.

During Prohibition some of this alcohol was diverted and used for illegal purposes. The analyst must be aware that the problem of diversion may not be completely solved. Consequently in suspicious cases, the possibility of a food product containing alcohol being contaminated with one of the above mentioned denaturants must not be completely overlooked.

Methods for the detection of many of these denaturants are given in the subsequent text. For the identification of alkaloids the reader is referred to an appropriate text. Inorganic materials may be detected as in any food.

Detection of Alcohol

During Prohibition it was often necessary to detect the presence of alcohol, in order to ascertain whether a near-beer or a so-called non-alcoholic wine was the product so stated. This is not necessary, unless called for by certain States. The presence of alcohol may be demonstrated by performing the following tests, generally on the distillate in order to concentrate the alcohol, if any is present, and also to eliminate interfering substances.

Iodoform Test—To the first 5 cc. of the distillate add 10 drops of 10 per cent sodium hydroxide solution and then add a solution of iodine in potassium iodide drop by drop, until a faint permanent yellow color is obtained, showing a slight excess of iodine. Allow the solution to stand for several minutes, shake and note the formation of crystals or the odor of iodoform. If no postive result is obtained in the cold, heat the tube to 60° C. for 1 minute and again allow to stand. A yellow crystalline precipitate of iodoform indicates the presence of ethyl alcohol. This reaction is not specific for it is given by a number of substances having the $CH_3.CH(OH).C$ or $CH_3.CO.C$ groups. Thus for example acetone and lactic acid give this reaction.

Ethyl Benzoate Test—To another small portion of the first part of the distillate add a few drops of benzoyl chloride and a few cc. of 10 per cent sodium hydroxide solution, and warm. The formation of the fruity ethyl benzoate with its characteristically smelling odor confirms the presence of ethyl alcohol. Acetone and lactic acid do not give this reaction and methyl alcohol, which also forms a characteristic odorous compound, does not give the iodoform reaction.

Estimation of Alcohol

The determination of alcohol in any alcoholic beverage is performed in a similar manner for all, except for minor variations depending on whether alcohol by volume or by weight is desired, and as to whether a beverage high or low in alcohol content is being analyzed. For materials low in alcohol content such as beer, cider and some wines, measure 100 cc. of the material by means of a pipette into a 300 to 500 cc. distillation flask. Add 50 cc. of water and attach the flask to a vertical condenser by means of a Polenske trap or some similar arrangement. For distilled liquors and similar products having a high alcohol content, measure 25 cc. of the product into the distillation flask, add 100 to 125 cc. of water and attach to the condenser as previously described. In the case of wines or other products having a high percentage of volatile acids, neutralize with sodium hydroxide before distillation. Distill over almost 100 cc. catching the distillate in a 100 cc. volumetric flask. Adjust the temperature to 20° C., make to volume, and determine the specific gravity of the distillate at that temperature by means of a pyknometer. In the case where 100 cc. of the sample was used, obtain the corresponding percentage of alcohol by volume, by reference to the alcohol table, Table

4 appendix. In the case of the 25 cc. sample, obtain the corresponding percentage of alcohol by volume, by reference to the alcohol table, and multiply the figure so obtained by 4 to yield the percentage of alcohol by volume in the original material. The alcohol content may be checked by determining the refractive index with the aid of an immersion refractometer and again referring to Table 4 appendix.

Analysis of Distilled Liquors

In general certain determinations may be made on distilled liquors in a manner entirely analogous to those we have previously encountered. Among these estimations may be mentioned, specific gravity, ash, acidity, metals, color and preservatives. However, other determinations are necessary at times before any conclusion as to the purity or genuineness of a distilled liquor may be ascertained.

Extract—Actually, the term extract is another designation for total solids not volatile. Evaporate 100 cc. of the sample, in a tared dish, to dryness, on a water bath, and dry in a constant temperature oven at 100° C. for 2.5 hours. The weight of the residue in the case of a whiskey should not exceed 0.5 g., nor should the residue be sticky, sweet or bitter. The former might indicate glycerol or sugar and the latter might indicate alkaloids. An indication of storage in wood barrels may be obtained from the extract by the addition of 25 cc. of water. The residue should not dissolve completely and the filtrate should give a greenish-black color on the addition of a drop of 10 per cent ferric chloride solution to 5 cc. of the filtrate.

Acidity—Dilute 50 cc. of the sample with 100 cc. of water and titrate with 0.1 N sodium hydroxide solution, using phenolphthalein as indicator. The result may be expressed in terms of acetic acid, 1 cc. of the alkali = 0.0060 g. of acetic acid. Whiskey should generally require not less than 4 cc. nor more than 12 cc. of the alkali solution.

Esters—Transfer by means of a pipette 100 to 200 cc. of the sample into a distillation flask and add 25 cc. of water. Distill very slowly 100 or 200 cc. according to the amount of the sample taken, and catch the distillate in an appropriate volumetric flask, taking care to have the tip of the condenser well within the neck of the flask, or else have the tip of the condenser extend through a two hole stopper inserted in the flask,

the other hole having a U-tube with a small amount of mercury in the bend to act as a mercury valve. These precautions are necessary to prevent loss. Neutralize 50 cc. of the distillate with 0.1 *N* sodium hydroxide solution. Add a measured amount of the standard alkali from 25–50 cc. according to the quantity of esters and boil for 10–30 minutes under a reflux condenser. Cool and titrate the excess alkali with 0.1 *N* hydrochloric or sulfuric acid. Calculate the number of cc. of the standard alkali used in the saponification of the esters as ethyl acetate basing the calculation on the relationship 1 cc. of 0.1 *N* sodium hydroxide solution is equivalent to 0.0088 g. of ethyl acetate. Whiskey should require not less than 2 cc. nor more than 8 cc. of 0.1 *N* alkali, on 100 cc. basis.

Aldehydes—This method is based on the restoration of the color of fuchsin in a fuchsin-sulfite solution. The residual aldehydes of the alcohol reagent is removed by the use of *m*-phenylenediamine hydrochloride. The A. O. A. C. gives the following directions:

Aldehyde-free alcohol: Redistill 95 per cent alcohol over sodium hydroxide or potassium hydroxide, add 2–3 g. per liter of *m*-phenylenediamine hydrochloride, digest at ordinary temperature for several days, or under a reflux condenser on a steam bath for several hours, and distill slowly, rejecting the first 100 cc. and the last 200 cc. of the distillate.

Sulfite-fuchsin solution: Dissolve 0.50 g. of pure fuchsin in 500 cc. of water, add 5 g. of sulfur dioxide dissolved in water, make up to 1 liter, and allow to stand until colorless. As this solution decomposes rapidly, prepare it in small quantities and keep at a low temperature.

Standard acetaldehyde solution: Prepare according to the directions of Vasey,[2] as follows: Grind aldehyde ammonia in a mortar with anhydrous ether and decant the ether. Repeat this operation several times and dry the purified salt in a current of air and then in vacuo over sulfuric acid. Dissolve 1.386 g. of this purified aldehyde ammonia in 50 cc. of 95 per cent alcohol, add 22.7 cc. of *N* alcoholic sulfuric acid, make up to 100 cc. and add 0.8 cc. of alcohol for the volume of the ammonium sulfate precipitate. Allow the mixture to stand overnight, and filter. This solution contains 1 g. of acetaldehyde in 100 cc. and will retain its strength.

The standard found most convenient for use is 2 cc. of this strong aldehyde solution diluted to 100 cc. with alcohol, 50 per cent by volume.

[2] Vasey, "Guide to the Analysis of Potable Spirits," Baillère, Tyndall and Cox (1904).

One cc. of this solution equals 0.0002 g. of acetaldehyde. Make up the solution every day or so, as it loses strength.

Determine the aldehyde in the distillate from the ester determination. Dilute 5–10 cc. of the distillate to 50 cc. with aldehyde-free alcohol, 50 per cent by volume; add 25 cc. of the sulfite-fuchsin solution, and allow to stand for 15 minutes at 15° C. The solutions and reagents should be at 15° C. when they are mixed. Prepare standards of known strength and blanks in the same way. The comparison standards found most convenient for use contain 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 mg. of acetaldehyde.

Denigès Reaction—To 2 cc. of the distillate from the ester determination, add 3 cc. of water and 10 cc. of a solution of mercuric sulfate, made by dissolving 5 g. of yellow mercuric oxide by mixing the oxide with 40 cc. of water, adding 20 cc. of sulfuric acid and then adding another 40 cc. of water and stirring until completely dissolved. Heat the mixture on a boiling water bath for 3 minutes. A precipitate indicates the possible presence of acetone, other ketones, isopropyl alcohol and tertiary butyl alcohol.

Acetone—Acetone may be determined in fermentation liquors by the *Messinger Method*.[3] Transfer 25 cc. of N sodium hydroxide to a 250 cc. glass-stoppered bottle, add 25 cc. of the distillate from the ester determination and then 35 cc. of 0.1 N iodine solution.[4] Shake constantly during the addition of the iodine solution and allow to stand for 15 minutes. Add 26 cc. of N hydrochloric acid solution and titrate the excess iodine with 0.1 N sodium thiosulfate solution using 1 cc. of 1 per cent starch solution as indicator. Run a blank determination on the same quantity of reagents, and deduct the number of cc. of 0.1 N iodine solution used in the blank from that consumed in the determination. Each cc. of 0.1 iodine solution = 0.0009675 g. of acetone.

Furfural—Dilute 10–20 cc. of the ester distillate to 50 cc. with furfural-free alcohol, 50 per cent by volume. Add 2 cc. of colorless aniline and 0.5 cc. of hydrochloric acid (sp. gr. 1.125) and allow to stand for 15 minutes, in a water bath. Compare with standards prepared from a solution of 1 g. of redistilled furfural in 100 cc. of 95 per cent alcohol

[3] Goodwin, *J. Am. Chem. Soc.* 42, 39 (1920).

[4] Stahly, Osburn and Werkman, *Analyst* 59, 319 (1934).

after diluting 1 cc. of this solution to 100 cc. with alcohol, 50 per cent by volume.

Methyl Alcohol—The methyl alcohol in the ester distillate is oxidized to formaldehyde and is then detected with fuchsin-sulfite solution, that is, the Schiff reagent. This reagent may be prepared by dissolving 0.5 g. of pure fuchsin in 400 cc. of warm water. Cool, add 2 g. of anhydrous sodium bisulfite and stir till dissolved. Add 4 cc. of sulfuric acid. Transfer to a brown bottle, allow to stand at least overnight before use. This reagent will not deteriorate over long periods if kept in a refrigerator.

As a check, a test for formaldehyde should be made on a portion of the distillate before oxidation with permanganate. This colorimetric test for methyl alcohol should be interpreted with caution when materials are being distilled that are likely to give derived methyl alcohol due to the splitting off of methoxyl groups, as for example, methyl alcohol derived from pectins in cider.

Transfer 0.5 cc. of the ester distillate to a test tube and dilute with 4.5 cc. of water. Add 2 cc. of a freshly prepared aqueous solution containing 3 g. of potassium permanganate and 15 cc. of phosphoric acid per 100 cc. and allow the mixture to stand for 10 minutes. Add 2 cc. of a solution containing 5 g. of oxalic acid dissolved in 100 cc. of a cooled mixture of equal volumes of sulfuric acid and water. When the solution is decolorized, add 5 cc. of the Schiff reagent prepared as directed above and mix well. Allow to stand for 10 minutes. The presence of methyl alcohol is indicated by the production of a blue or violet color. No other aldehydes interfere because the production of color by other aldehydes is inhibited by the addition of acid.

Methyl alcohol may be quantitatively determined by a modification of the above test.[5] Transfer 10 cc. of the methyl alcohol solution to a 25 cc. volumetric flask and add 1 cc. of 5 per cent ethyl alcohol by volume. Add 1 cc. of 1 per cent potassium permanganate and then 1 cc. of phosphoric acid solution, 25 cc. of 85 per cent phosphoric acid in 100 cc. water. Allow the mixture to stand for 1 hour with occasional shaking. Add 1 cc. of 5 per cent oxalic acid solution. When the mixture is colorless, add 2 cc. of sulfuric acid solution, 30 cc. sulfuric acid in 100 cc. water. Then add 5 cc. of the Schiff reagent. After 3 hours, compare in a colorimeter against a standard treated exactly the same way at the same time.

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[5] Jephcott, *Analyst* 60, 714 (1935).

This method holds for 30 parts per million, although 5 parts per million may be detected qualitatively. The color produced is not strictly proportional to the concentration. However, in the range from 100 parts per million to 250 parts per million, the color is apparently a straight line function of the concentration. Consequently it is best to keep quantitative determinations within this range.

Immersion Refractometer Method. This method is best used for larger quantities of methyl alcohol in ethyl alcohol. Small quantities of methyl alcohol are preferably estimated colorimetrically. Determine the refractive index of the distillate obtained in the alcohol determination by means of an immersion refractometer as detailed in Chapter II. If, on referring to Table 4 appendix, on scale readings of the immersion refractometer, the refractive index corresponds with the percentage alcohol obtained by means of specific gravity determinations, it may be assumed that no methyl alcohol is present. If on the other hand the refractometer reading is low, an appreciable amount of methyl alcohol is present, provided no other refractive substances other than water and the alcohols are present in significant amounts. Methyl alcohol decreases the refraction of ethyl alcohol in direct proportion to the amount of methyl alcohol present, hence, a quantitative calculation may be made by interpolation in Table 52 of the figures for pure ethyl and methyl alcohol of the same alcoholic strength—as the sample being used.

For example, if the distillate has a specific gravity of 0.9791 at 20° C./20° C. and an immersion refractometer reading of 26.8 at 20° C., then the percentage of methyl alcohol may be estimated as follows: The specific gravity found corresponds to 13.29 per cent by weight of ethyl alcohol as shown in Table 4 appendix. According to Table 52, 13.29 per cent of ethyl alcohol corresponds to a scale reading of 37.4. The scale reading corresponding to this per cent of methyl alcohol would be 22.2. The difference between these scale readings is $37.4 - 22.2 = 15.2$. The difference between the ethyl alcohol calculated refractometer scale reading and the observed refractometer scale reading is $37.4 - 26.8 = 10.6$. Then dividing the observed difference by the calculated difference, $10.6/15.2 = 69.7$ per cent methyl alcohol.

A more exact calculation may be made by obtaining the percentage of methyl alcohol by weight corresponding to the gravity given above. Then referring to Table 52 obtain the appropriate scale reading. This should then be subtracted from the ethyl alcohol reading and the remaining calculation performed as explained above.

TABLE 52. SCALE READINGS OF IMMERSION REFRACTOMETER OF METHYL AND ETHYL ALCOHOLS AT 20° C.[6]

Per Cent Alcohol by Weight.*	Scale Readings at 20° C.		Per Cent Alcohol by Weight.	Scale Readings at 20° C.		Per Cent Alcohol by Weight.	Scale Readings at 20° C.	
	Methyl Alcohol.	Ethyl Alcohol.		Methyl Alcohol.	Ethyl Alcohol.		Methyl Alcohol.	Ethyl Alcohol.
0	14.5	14.5	15.5	23.55	41.5	31	33.5	70.4
0.5	14.65	15.25	16	23.9	42.5	31.5	33.8	71.05
1	14.8	16.0	16.5	24.2	43.5	32	34.1	71.7
1.5	15.1	16.8	17	24.5	44.5	32.5	34.4	72.4
2	15.4	17.6	17.5	24.85	45.5	33	34.7	73.1
2.5	15.7	18.35	18	25.2	46.5	33.5	34.95	73.75
3	16.0	19.1	18.5	25.5	47.5	34	35.2	74.4
3.5	16.3	19.9	19	25.8	48.5	34.5	35.5	75.1
4	16.6	20.7	19.5	26.15	49.5	35	35.8	75.8
4.5	16.9	21.5	20	26.5	50.5	35.5	36.05	76.35
5	17.2	22.3	20.5	26.8	51.45	36	36.3	76.9
5.5	17.5	23.2	21	27.1	52.4	36.5	36.55	77.95
6	17.8	24.1	21.5	27.45	53.35	37	36.8	78.0
6.5	18.1	25.0	22	27.8	54.3	37.5	37.05	78.55
7	18.4	25.9	22.5	28.1	55.3	38	37.3	79.1
7.5	18.7	26.85	23	28.4	56.3	38.5	37.5	79.65
8	19.0	27.8	23.5	28.75	57.25	39	37.7	80.2
8.5	19.3	28.7	24	29.1	58.2	39.5	37.9	80.75
9	19.6	29.6	24.5	29.4	59.15	40	38.1	81.3
9.5	19.9	30.5	25	29.7	60.1	40.5	38.25	81.8
10	20.2	31.4	25.5	30.0	61.0	41	38.4	82.3
10.5	20.5	32.3	26	30.3	61.9	41.5	38.6	82.8
11	20.8	33.2	26.5	30.6	62.8	42	38.8	83.3
11.5	21.1	34.1	27	30.9	63.7	42.5	39.0	83.75
12	21.4	35.0	27.5	31.25	64.6	43	39.2	84.2
12.5	21.7	35.95	28	31.6	65.5	43.5	39.25	84.7
13	22.0	36.9	28.5	31.9	66.35	44	39.3	85.2
13.5	22.3	37.8	29	32.2	67.2	44.5	39.35	85.7
14	22.6	38.7	29.5	32.4	68.1	45	39.4	86.2
14.5	22.9	39.6	30	32.8	69.0	45.5	39.45	86.6
15	23.2	40.5	30.5	33.15	69.7	46	39.5	87.0

[6] Van Nostrand's Chemical Annual, 7th issue, pp. 666-7 (1934).

TABLE 52.—*Continued*

Per Cent Alcohol by Weight. *	Scale Readings at 20° C.		Per Cent Alcohol by Weight.	Scale Readings at 20° C.		Per Cent Alcohol by Weight.	Scale Readings at 20° C.	
	Methyl Alcohol.	Ethyl Alcohol.		Methyl Alcohol.	Ethyl Alcohol.		Methyl Alcohol.	Ethyl Alcohol.
46.5	39.55	87.4	64.5	35.75	98.15	82.5	23.45	100.45
47	39.6	87.8	65	35.5	98.3	83	23.6	100.4
47.5	39.65	88.25	65.5	35.25	98.5	83.5	23.2	100.35
48	39.7	88.7	66	35.0	98.7	84	22.8	100.3
48.5	39.75	89.1	66.5	34.75	98.9	84.5	22.3	100.2
49	39.8	89.5	67	34.5	99.1	85	21.8	100.1
49.5	39.8	89.9	67.5	34.25	99.25	85.5	21.3	99.95
50	39.8	90.3	68	34.0	99.4	86	20.8	99.8
50.5	39.75	90.7	68.5	33.75	99.55	86.5	20.25	99.65
51	39.7	91.1	69	33.5	99.7	87	19.7	99.5
51.5	39.65	91.45	69.5	33.25	99.85	87.5	19.15	99.35
52	39.6	91.8	70	33.0	100.0	88	18.6	99.2
52.5	39.6	92.1	70.5	32.65	100.1	88.5	17.95	99.05
53	39.6	92.4	71	32.3	100.2	89	17.3	98.9
53.5	39.55	92.7	71.5	32.0	100.3	89.5	16.7	98.75
54	39.5	93.0	72	31.7	100.4	90	16.1	98.6
54.5	39.45	93.3	72.5	31.4	100.5	90.5	15.5	98.45
55	39.4	93.6	73	31.1	100.6	91	14.9	98.3
55.5	39.3	93.85	73.5	30.75	100.7	91.5	14.3	98.05
56	39.2	94.1	74	30.4	100.8	92	13.7	97.8
56.5	39.1	94.4	74.5	29.75	100.9	92.5	13.05	97.5
57	39.0	94.7	75	29.7	101.0	93	12.4	97.2
57.5	38.8	94.95	75.5	29.35	101.0	93.5	11.7	96.8
58	38.6	95.2	76	29.0	101.0	94	11.0	96.4
58.5	38.45	95.45	76.5	28.65	100.95	94.5	10.3	96.05
59	38.3	95.7	77	28.3	100.9	95	9.6	95.7
59.5	38.1	95.55	77.5	27.95	100.9	95.5	8.9	95.3
60	37.9	96.2	78	27.6	100.9	96	8.2	94.9
60.5	37.7	96.45	78.5	27.2	100.85	96.5	7.45	94.45
61	37.5	96.7	79	26.8	100.8	97	6.7	94.0
61.5	37.25	96.9	79.5	26.4	100.75	97.5	5.9	93.5
62	37.0	97.1	80	26.0	100.7	98	5.1	93.0
62.5	36.75	97.3	80.5	25.55	100.65	98.5	4.3	92.5
63	36.5	97.5	81	25.1	100.6	99	3.5	92.0
63.5	36.25	97.75	81.5	24.7	100.55	99.5	2.75	91.5
64	36.0	98.0	82	24.3	100.5	100	2.0	91.0

Diethylphthalate—If the substance to be analyzed contains a considerable quantity of diethylphthalate, the following procedure may be used. To 10 cc. of the sample add 1 cc. of a 10 per cent sodium hydroxide solution and evaporate to dryness on a steam bath. To the dry residue add 5 cc. of sulfuric acid and warm for several minutes on the bath. Add 25 mg. of resorcinol and again heat for several minutes. Transfer the liquid to a test tube and heat to 160° C. for 5 minutes, using a paraffin bath. When cool, pour into 200 cc. of water and make alkaline. The characteristic yellow-green fluorescence will appear and is indicative of fluorescein derived from phthalic acid.

For small quantities of diethylphthalate proceed as follows: To 50 cc. of the liquid sample in a porcelain dish or casserole add 0.2 cc. of 10 per cent sodium hydroxide and evaporate to dryness on a steam bath. If necessary the residue may be heated in a thermostatically controlled electric oven at 125° C., to obtain absolute dryness. To this residue add 5 cc. of sulfuric acid and warm on the steam bath for several minutes. Add not more than 25 to 40 mg. of resorcinol and again warm the mixture until solution is effected. Transfer the mixture to a test tube and heat in a paraffin bath at 160–170° C. for 10 minutes. Pour the cooled melt into 150 cc. of water and make alkaline with 10 per cent sodium hydroxide. If diethylphthalate is present in the sample, the characteristic greenish-yellow fluorescence of fluorescein is at once apparent when viewed against a black background.

Extreme care should be taken to have clean apparatus in running the test. A blank should be run to insure proper interpretation of results. In all cases where a slight but decided fluorescence is obtained the solution should be allowed to stand for several days to observe any fading. Twenty-four hours should be allowed in every test to permit any pseudo-fluorescence to disappear.

Isopropyl Alcohol—Isopropyl alcohol and other substances such as acetone may be detected by the Denigès reaction. In the absence of acetone isopropyl alcohol may be confirmed by the following test. Place 8 g. of chromic acid, CrO_3, in a Kohlrausch flask of about 100 cc. capacity or some other suitable flask and add 15 cc. of water and about 2 cc. of sulfuric acid. Connect the flask with a reflux condenser and add very slowly through the condenser 5 cc. of the sample to be tested. The reaction is very vigorous and causes the solution to boil. Continue boiling under the reflux condenser for about one-half hour; cool and distill, without transferring the liquid until 2 cc. have been collected, using a 10 cc.

graduated cylinder as a receiver. Mix the distillate and test for acetone in the usual way with Denigès reagent, that is, to 2 cc. of Denigès reagent add 4 to 5 drops, not more of the distillate and bring to a boil, keeping the solution at just about the boiling temperature about 1 minute or slightly longer. A fine white precipitate that does not settle rapidly is indicative of acetone, which was obtained from the oxidation of isopropyl alcohol. Any precipitate formed after heating 2 minutes and allowed to cool should be disregarded. Acetaldehyde, paraldehyde, ethyl acetate, acetic acid and mixtures of these do not interfere.

In case acetone is present it may be determined quantitatively as described under the section, "Acetone," Chapter XIV, in an aliquot of the distillate from the fermentation liquid. In another aliquot the isopropyl alcohol is oxidized to acetone and the total acetone determined as before. It has been found that only 94 per cent of isopropyl alcohol is quantitatively oxidized to acetone.

Fusel Oil—*Allen*[7]*-Marquardt*[8] *Method.* Saponify 50 cc. of the sample by boiling for one hour under a reflux condenser or by allowing to stand overnight with the addition of 50 cc. of water and 20 cc. of 0.5 *N* sodium hydroxide solution. Connect the flask by means of a Polenske trap to an upright condenser and distill directly 90 cc. Add an additional 25 cc. to the flask and continue the distillation until 25 cc. more is collected.

If aldehydes are present in excess of 15 parts in 100,000, add to the distillate 0.5 g. of *m*-phenylenediamine hydrochloride, and boil under a reflux condenser for one hour. Connect the flask as directed above, and distill over 100 cc. Add 25 cc. of water to the flask and continue the distillation until an additional 25 cc. is collected.

Approximately saturate the distillate with powdered sodium chloride and add saturated sodium chloride solution until the specific gravity is 1.10, which may be determined by a hydrometer. Extract the salt solution 4 times with purified carbon tetrachloride, using 40, 30, 20 and 10 cc. of the solvent successively. Wash the combined carbon tetrachloride extracts 3 times with 50 cc. portions of saturated sodium chloride solution and twice with saturated sodium sulfate solution. Transfer the carbon tetrachloride to a flask containing 50 cc. of a solution of 100 g. of potassium dichromate in 900 cc. of water and 100 cc. of sulfuric acid, and boil for

[7] Allen and Chattaway, *Analyst* **9,** 102 (1891).
[8] Marquardt, *Ber.* **15,** 1370 (1882).

8 hours under a reflux condenser. Add 100 cc. of water and distill until only about 50 cc. remains. Add 50 cc. of water to the flask and again distill until 35 to 50 cc. is left. Use care to prevent the oxidizing mixture from caking on the side of the distilling flask. The distillate should be water white. If it is colored, discard and repeat the determination. Titrate the distillate with 0.1 *N* sodium hydroxide solution using phenolphthalein as indicator. 1 cc. of 0.1 *N* sodium hydroxide solution is equivalent to 0.0088 g. of amyl alcohol. Corks covered with tinfoil should be used in the distillation from the oxidizing mixture and preferably all glass apparatus should be used.

Run a blank on 100 cc. of carbon tetrachloride beginning the blank with the washing with the salt solutions.

The higher alcohols are extracted from the saturated salt solution by carbon tetrachloride and are subsequently oxidized by means of the potassium dichromate-sulfuric acid solution. The volatile fatty acids formed are then distilled and estimated by titration with 0.1 *N* alkali solution. Interfering substances such as esters and aldehydes are removed by the prior saponification and the addition of *m*-phenylenediamine.

Fusel oil is not only composed of amyl alcohol, but also contains some isopropyl and isobutyl alcohol, which are oxidized to acetone and butyric acid respectively. The former is obviously not included in the alkali titration and therefore the result, for fusel oil content, obtained by this method is undoubtedly low.

Colorimetric method: It is well known that the Allen-Marquardt method recovers only 60–70 per cent of the higher alcohols. Other methods such as shaking the sample with a measured volume of chloroform and noting the increase in volume of the extract as due to fusel oil are also in error. Penniman, Smith and Lawshe [9] developed a method based on the removal of interfering aldehyes, saponification and recovery of the higher alcohols by distillation, production of unsaturated hydrocarbons with sulfuric acid and subsequent colorimetric estimation of the fusel oil by color reagents.

Place 25 cc. of sample in a 500 cc. round-bottomed flask. Add 0.5 g. of silver sulfate and 1 cc. of sulfuric acid (1 : 1), and make the total volume up to 110 cc. Reflux gently for 15 minutes. Bumping can be prevented by the addition of small quantities of granulated zinc. If foaming occurs, this can be reduced by adding 15 g. of sodium chloride. The addition of salt results in a partial conversion of silver oxide to

[9] Penniman, Smith and Lawshe, *Ind. Eng. Chem., Anal. Ed.* 9, 91 (1937).

silver chloride, but this fact does not appear to affect the efficiency of the silver as a dealdehyding agent.

After saponification, the sample is distilled, 75 cc. of the distillate being collected. This distillate contains all of the higher alcohols originally present in the sample. The concentration has been reduced to one-third of that of the sample. This reduction in the concentration is made necessary by the extreme sensitivity of the color reagents.

Procedure with p-dimethylaminobenzaldehyde or salicylaldehyde reagent: Place 2 cc., delivered with an accurate pipette, of the distillate in a 125 cc. Florence flask. Add 20.0 cc. of sulfuric acid, swirling the flask in a bath of cold water during the addition. Then add exactly 2 cc. of a solution of the reagent in 95 per cent alcohol, 10 mg. per cc., again swirling the flask in cold bath.

Prepare a similar flask containing 2 cc. of a standard fusel oil solution, acid, and reagent.

Place the flasks simultaneously in a bath of vigorously boiling water. After 20 minutes, transfer the flasks to the cold bath. When cool add 25 cc. of sulfuric acid (1:1) and mix thoroughly by swirling. Compare in a colorimeter of the Duboscq type or other suitable colorimeter.

Procedure with Vanillin reagent: Proceed as directed above for the other two reagents, except for the following differences: a) use only 10 cc. of sulfuric acid in making up the reaction mixture; b) the vanillin solution contains 17.5 mg. of the reagent per cc. of 95 per cent alcohol.

Because the colors produced are not entirely directly proportional to the fusel oil concentration, it is necessary to compare the sample with a standard of approximately equal fusel oil content. If standard fusel oil made from sweet-mash rye whiskey of the Maryland type or similar standard fusel oils are not available, a satisfactory standard can be made by mixing isoamyl (b.p. 132) and isobutyl alcohols in the ratio of 4 to 1. The synthetic mixture is slightly weaker in color value than fusel oil, the ratio being 0.95 for p-dimethylaminobenzaldehyde or salicylaldehyde and 0.90 for vanillin. In using the synthetic standard, the colorimeter reading should be multiplied by the appropriate standard.

The concentrations and times must be followed rigidly for the reaction is not allowed to go to completion but is arbitrarily stopped after a definite length of time by chilling the reaction mixture and diluting. It is therefore necessary to run sample and standard together.

Benzene—If benzene is suspected in what one might call relatively large amounts, it may readily be detected by the turbidity that would

be caused in the liquor or distillate on dilution with water. If the amount present is too small to yield a turbidity, indications of its presence may be obtained through taste and odor. Lansing[10] suggests a procedure involving the concentration of the benzene by extraction, nitration of the extract, and a color reaction with the nitration product in order to detect low concentrations of benzene.

Reagents: 1) Amyl alcohol.—Redistilled crude fusel oil refluxed with strong caustic soda solution, and distilled from the alkali, collecting for use the dry fraction boiling at 128–132° C.

2) Nitrating acid.—70 g. of reagent 20% oleum, 45 g. of sulfuric acid (sp. gr. 1.84) and 43 g. of nitric acid (sp. gr. 1.42).

3) Caustic Soda solution.—140 g. of reagent sticks made up to 250 cc. at room temperature.

Determination: If the distillate contains much above 0.01 per cent benzene by volume, as indicated by test on the undiluted sample, it is diluted to approximately that value with alcohol free from benzene. A 40 cc. portion is placed in a 100 cc. glass-stoppered cylinder with 6 cc. of carbon tetrachloride. Water is added to the 90 cc. mark, followed by 10 cc. of a solution of 10 g. of anhydrous sodium sulfate made up to 100 cc. with water. The cylinder is stoppered, shaken thoroughly, its contents are transferred to a separatory funnel and allowed to stand until the layers separate sharply. The lower layer is drawn off and 5 cc. of the layer is transferred by pipette to a test tube. Three cc. of the nitrating acid is measured in a small cylinder and added to the tube, which is shaken carefully but thoroughly. During 10 minutes the tube is shaken twice more, and at the end of that time, 20 cc. of water is added, preferably rapidly from a cylinder. After mixing by pouring into another test tube and back again, the bulk of the water layer is decanted and discarded. The lower layer with a small amount of water layer is transferred to an evaporating dish placed on a hot plate. When the carbon tetrachloride has evaporated, the dish is emptied into the same test tube previously used, and is rinsed with 1 cc. of amyl alcohol. The dish is then rinsed with 4 cc. of caustic soda solution. The rinsings are added to the test tube. The tube is mixed by swirling and then 1 cc. of acetone is added. The tube is swirled again, and placed in a rack for observation of the color produced in the top layer.

Benzene, 1 part in 10,000 by volume, in a rectified alcohol of ordinary purity gives a red color with a purple quality by this method. This color

[10] Lansing, *Ind. Eng. Chem., Anal. Ed.* 7, 184 (1935).

holds for some hours, and finally fades to a dull orange-red. Concentrations much higher than that quoted yield colors too dark for proper identification. Toluene gives a brownish-yellow and xylene gives an intense green which fades to a dull orange in 30 minutes. Blank runs should be conducted with the test and give a light clear lemon yellow.

Caramel—Imitation or artificial whiskies and some blended whiskies are colored at times with caramel or coal tar colors. Adulterated whiskies may, of course, also have artificial color. Caramel and some coal tar colors may be detected by the Marsh test. For a complete examination of the coloring matters, the methods detailed in Chapter III must be used.

Dilute 10 cc. of the sample with 2 cc. of water and add 15 cc. of the Marsh reagent, which is composed of 100 cc. of pure amyl alcohol, 3 cc. of syrupy phosphoric acid and 3 cc. of water. Shake the mixture gently for 2 minutes and allow the layers to separate completely. The lower aqueous layer should be colorless or nearly colorless. A colored aqueous layer indicates the presence of caramel or some coal tar colors. These colors may be identified by the scheme suggested in Chapter III.

Alkaloids—Make 10 cc. of the sample acid with 5 drops of dilute hydrochloric acid and evaporate to 5 cc. Dilute with water to 10 cc. and filter. Add a few drops of 0.1 *N* sodium hydroxide solution. In the presence of alkaloids a precipitate will develop.

For identification, the alkaloids must be extracted with the aid of immiscible solvents and then may be identified by means of microchemical tests. The reader is referred to books dealing with alkaloids.

Artificial Aging of Spirits

The aging of spirits involves oxidation. It is this reaction that is hastened by the processes devised to accelerate aging. Fain and Snell [11] classify the methods for aging spirits artificially into 4 main classes.

1) treatment with air, oxygen, or ozone
2) exposure to actinic rays
3) electrolytic treatment
4) use of catalysts

There are of course combinations of these methods.

[11] Fain and Snell, *Ind. Eng. Chem., News Ed.* 12, 120 (1934).

An example of type 1 provides for the treatment of the liquor with oxygen, while exposed on large wooden surfaces which have been impregnated with a solution obtained by extracting sea weed ash. Type 2 may be illustrated by the method in which wines and liquors in a thin layer are acted upon by ultra violet light from a mercury vapor electric arc. In type 3, the beverages are artificially aged by an electrolytic treatment producing hydrogen and oxygen in the liquid. The electrodes and the diaphragm between them are impregnated with insoluble inorganic salts or oxides capable of producing oxidation and reduction effects in the presence of the oxygen and hydrogen produced. Sometimes oak shavings, called "toasted chips" are added to the spirits during the treatment and the electrolysis due to cataphoresis, will assist in the extraction of materials from the oak chips. In type 4 the vapors are passed over finely dispersed metallic oxides such as those of copper, nickel and titanium at 150° to 180° C. Fain and Snell [11] discuss some of these processes more fully and give a bibliography.

Beavens, Goresline and Nelson [12] use silver in the artificial aging of spirits. This process consists of dispersing minute quantities of ionic silver in the brandy. Brandies given this treatment and held for a week showed a mellowing effect and considerable change in flavor and aroma. The method may be useful for the quick aging of brandies, and although there is a change in the flavor and aroma, apparently no significant change, as indicated by chemical analysis, takes place in the acid, ester, aldehyde or fusel oil content of the brandy.

Interpretation of Results—Distilled Liquors

A distilled liquor may be regarded as an alcohol-water solution containing small quantities of secondary constituents which usually amount to less than 1 per cent of the whole. The secondary constituents which impart to the beverage its characteristic bouquet may be divided into 3 groups; a) substances derived from the original grain or other starting material by the process of fermentation, distillation, etc.; b) reaction products formed during aging, particularly as a result of esterification and oxidation and c) flavor-producing substances added or extracted from wood. The first group consists chiefly of higher alcohols and esters. The higher alcohols are grouped together under the term, fusel oil. Taking into consideration the newer methods of quick-aging, a fourth group

[12] Beavens, Goresline and Nelson, *Ind. Eng. Chem.* 29, 623 (1937).

d) may be classified as those substances added to effect the quick aging.

An exhaustive investigation of the change in the composition of whiskey on storage over a period of years was made by Crampton and Tolman.[13] They found that water passes more easily than alcohol through the pores of the wood barrels, consequently the alcoholic strength increased with age. The increase in the amount of higher alcohols and other characteristic substances found in distilled liquors is due to the fact that wood is impervious to these materials. The increase in extract, acids, esters, aldehydes, furfural and fusel oil is, however, not only due to retention, but also to the chemical changes that the distilled materials undergo while being stored in wood. If the wood is charred these changes are much greater.

Rye whiskey generally has higher constants for the above-mentioned characteristics than bourbon, because it is stored in heated warehouses. The heat, as usual, speeds the chemical reactions.

In new whiskies the acid content is usually less than the ester content, but as the storage proceeds, the acids form more rapidly, then the process is reversed and the esters form more rapidly, the net result being, that after four years' storage, the acid and ester content of whiskies are approximately equal. High color, high solid content, and high alcohol concentration are generally accompanied by high acid and high ester content. The amounts of higher alcohol increase in the matured whiskey only in proportion to the alcohol concentration. The oily appearance of a matured whiskey is due to the material extracted from the charred container and this appearance is almost lacking in whiskies aged in uncharred barrels. In a new whiskey the furfural content is small, because the furfural is derived in small measure from the mash and, to a much larger extent, from the charred wood.

The above mentioned conclusions are mainly drawn from the work of Crampton and Tolman. Valaer and Frazier[14] conducted an investigation on the changes in whiskey stored for four years. Their conclusions differ somewhat from the equally comprehensive investigation of the aforementioned workers. The samples Valaer and Frazier investigated represented a variety of whiskies: two sweet-mash ryes, two sour-mash ryes, two sweet-mash bourbons and 5 sour-mash bourbons. Four of the samples had received quick age treatment. The whiskies were manufac-

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[13] Crampton and Tolman, *J. Am. Chem. Soc.* 30, 97 (1908).

[14] Valaer and Frazier, *Ind. Eng. Chem.* 28, 92 (1936).

tured in legitimate distilleries to replace medicinal stocks during the Prohibition period. They came to the following conclusions.

The largest increase of acids, esters, solids, and color is during the first 6 months of storage. The acids and esters did not reach a ratio of 1:1, and the analyses showed no fixed relationship between these two compounds as found by Crampton and Tolman, at the end of 4 years.

There is an actual as well as an apparent gain in acids over the 4-year period using the data calculated to original volume. The actual acid gains were from 24.9 to 56 g. per 100 liters with an average of 40.1 g. There is an actual as well as an apparent gain in esters over the 4-year period when calculated to original volume. The esters actually gained from 7.4 to 21.3 g. per 100 liters with an average of 15.5 g. There is an actual loss of fusel oil during the aging period, which is from 6.9 to 58.4 g. per 100 liters with an average loss of 28.6 g. when calculated to original volume.

Quick-aging process increased the acids, solids, color, and furfural at the start. The color, solids, and furfural maintained the ratio of increase until the end of the 4-year period.

Whiskey changes while standing in glass. There is usually a decrease in acids and a tendency for esters to increase and there is often an increase in color. Newly distilled whiskey stored in glass, however, has practically no change in chemical composition but undergoes a surprising change in taste and odor, losing all of its "slop" taste and odor.

They found that the constituents of whiskey are continually undergoing changes as the aging process progresses, in agreement with Crampton and Tolman.

The increase in acids in charred barrels is due partly to fixed acids extracted from the wood but mostly to the formation of volatile acids during the aging process.

Since esters are not produced by the ordinary quick-aging processes, these congenerics present a reliable index to the age of the whiskey. The method of analysis requires much care and the details must be followed implicitly if the results are to be considered of utmost value. The principal ester is ethyl acetate but no doubt esters of the higher alcohols are also present, since it is impossible to obtain the characteristic aroma or bouquet of aged whiskey with ethyl acetate alone.

A comparison then of the analyses of a distilled liquor with the authentic analyses in Tables 53 and 54 will aid in judging the authenticity of a sample. However, all of the determinations and their relationships must be considered before making a decision. The simple fact

that the constants of a whiskey fall between the limits shown by the maxima and minima figures, is alone no definite indication as to its genuineness. The detection of any of the adulterants mentioned previously, consistently low constants, ratios that are not normal, for example high esters compared to low acids in a whiskey presumed to be old, all aid in the conclusions to be drawn as to the purity of the sample.

TABLE 53. AVERAGE, MAXIMA, AND MINIMA DATA ON WHISKEY [15]

(Grams per 100 liters, 100 proof spirit)

Age	Proof		Color	Solids	Acids	Esters	Alde-hydes	Fur-fural	Fusel Oil
New	ave.	101.9	....	20.1	6.4	16.3	3.9	0.9	95.2
	max.	104.0	....	161.0	29.1	53.2	15.0	2.0	171.3
	min.	100.0	....	5.0	1.2	1.3	trace	trace	42.0
1 yr.	ave.	102.0	8.2	109.4	43.6	32.6	6.7	1.7	110.7
	max.	104.0	13.8	193.0	60.5	64.8	15.5	7.9	194.0
	min.	100.0	4.6	54.0	5.8	6.8	1.5	0.2	42.8
2 yrs.	ave.	103.6	10.1	135.0	48.6	46.6	9.3	1.8	114.0
	max.	109.0	16.7	214.0	63.0	75.1	18.7	9.1	214.0
	min.	100.0	5.7	78.0	11.0	11.2	5.9	0.4	42.8
3 yrs.	ave.	105.2	11.7	160.1	58.5	54.8	11.5	2.1	121.2
	max.	112.0	18.3	245.0	81.8	83.9	22.1	9.5	202.0
	min.	100.0	7.0	90.0	16.4	12.1	5.9	0.6	43.5
4 yrs.	ave.	107.6	12.4	167.9	62.2	61.1	12.4	2.3	125.8
	max.	118.0	18.9	249.0	83.8	89.1	22.2	9.6	237.1
	min.	100.0	7.4	92.0	17.3	13.8	6.4	0.7	43.5
5 yrs.	ave.	109.8	14.1	189.0	64.6	65.0	13.1	2.5	126.8
	max.	125.0	19.2	280.0	92.6	105.5	23.1	9.6	254.2
	min.	101.0	8.4	114.0	19.0	17.3	6.6	0.8	45.1
6 yrs.	ave.	112.8	15.0	203.5	69.7	69.5	13.1	2.6	139.9
	max.	132.0	21.2	287.0	96.8	109.0	23.7	9.5	245.3
	min.	102.0	9.3	132.0	24.3	17.9	7.5	0.7	44.6
7 yrs.	ave.	115.3	15.9	220.9	74.3	73.0	13.8	2.5	141.0
	max.	141.0	22.7	309.0	100.0	114.9	26.7	8.5	264.5
	min.	102.0	10.1	134.0	24.7	21.3	7.5	0.8	46.6
8 yrs.	ave.	117.0	16.3	231.6	79.4	76.6	14.3	2.7	148.8
	max.	134.0	24.2	326.0	112.0	126.6	28.8	10.0	280.3
	min.	102.0	10.5	141.0	31.7	22.1	7.9	0.8	47.6

15 Crampton and Tolman, *J. Am. Chem. Soc.* 30, 98 (1908).

TABLE 54. MINIMUM, AVERAGE AND MAXIMUM DATA ON WHISKEY SAMPLES[16]

(Grams per 100 liters calculated to proof)

Age years	Range Proof	Total acids	Esters	Fusel oil	Solids (extract)	Alde-hydes	Fur-fural[17]	Color in 0.5 inch cell
New[18]	min. 100.0	5.3	13.7	78.7	2.9	2.4	0	....
	ave. 101.2	7.7[19]	17.0	161.1	10.5	7.6	....	....
	max. 102.6	9.6	21.5[19]	230.7	20.1	20.8	....	....
0.5	min. 100.0	31.7	18.2	87.0	61.6	3.9	0.6	4.4
	ave. 101.3	40.3	26.5	166.8	92.5	9.6	1.7	7.1
	max. 102.9	52.4	32.8	244.3	121.9	22.7	2.2	9.9
1	min. 100.5	38.5[18]	21.9	92.0	89.8	4.3	0.6	6.3
	ave. 101.9	50.1	29.9	166.5	114.4	10.2	1.9	8.6
	max. 103.3	53.4[18]	35.3	245.4	135.1	20.5	2.2	12.2
1.5	min. 100.7	44.7	22.4	96.7	98.4	4.7	0.8	7.2
	ave. 102.8	55.9	32.4	167.0	131.3	10.4	1.9	9.8
	max. 104.0	62.8	38.2	240.4	160.7	21.8	2.2	13.6
2	min. 101.2	48.2	25.2	97.6[19]	113.7	4.6	0.8	7.7
	ave. 103.8	59.7	34.7	168.3	143.0	11.0	1.9	10.8
	max. 105.3	65.6	38.7	232.1	166.7	24.5	2.2	14.5
2.5	min. 101.9	51.4	28.3	96.6	125.0	4.6	0.8	8.6
	ave. 104.8	62.8	37.0	168.6	155.8	11.0	2.1	11.9
	max. 106.1	72.6	43.1[19]	237.7	183.1	22.7	2.4	15.6
3.0	min. 102.4	54.1	28.5	98.4[19]	129.8	4.6	0.8	9.4
	ave. 105.6	65.2	38.9	172.0	163.0	11.1	2.1	12.3
	max. 107.3	73.6	43.9[20]	249.8	197.9	22.6	2.7	15.7
3.5	min. 102.8	58.9	29.1	95.6	132.5	5.2	0.8	9.8
	ave. 106.6	67.9	40.0	171.2	172.8	11.2	2.1	13.1
	max. 109.0	74.8	46.2	241.3	207.4	22.1	2.9	16.1
4	min. 104	59.8[18]	37.6	96.0	141.8	6.0	0.8	10.2
	ave. 107.7	70.6	45.0	178.5	178.7	11.6	2.2	14.1
	max. 110.7	78.6	48.8[19]	260.8	213.8	21.7	3.0	17.3

[16] Valaer and Frazier, *Ind. Eng. Chem.* 28, 92 (1936).

[17] In the maximum furfural the quick age samples and those from one distillery which seemed abnormal were omitted.

[18] Does not include quick aged samples.

[19] Highest or lowest was omitted as being slightly abnormal, and the next highest was taken instead.

[20] Highest and next were omitted as being slightly abnormal.

Nevertheless, it must be admitted that the phrase, a scholar, a gentleman and a judge of good liquor, is not to be scorned in the estimate of liquor samples for a trained taste is as efficient in judging distilled liquors as in judging wine or tea. However, only a trained taste may be used. Mere consumption of large quantities of liquors does not make one qualify.

Analysis of Wines

According to McCharles and Pitman [21] the more important determinations to be made on wines are the determinations of alcohol, volatile acids with correction for sulfites, total acids, reducing sugars, metals, sulfur dioxide, and tannin. From the definitions of the Federal Government, we know that ash, chloride, and sulfate content are also important factors. Other estimations giving information are glycerol and extract.

Specific gravity, ash, sulfates, phosphates, chlorides, nitrates, reducing sugars, sucrose, commercial glucose, protein, dextrin, coloring matter, and preservatives including sulfites may be estimated in a manner entirely analogous to methods detailed in other sections of the book. The determination and detection of alcohol and other materials may be made as outlined in the preceding sections of this chapter.

Extract—The methods of the A. O. A. C. give the following details for the determination of extract in wine, by calculating the extract from the specific gravity of the dealcoholized wine. The wine may be dealcoholized by evaporation of the alcohol from a given volume on a bath and subsequently adjusted to the original volume. Calculate the specific gravity of the dealcoholized wine by the following formula:

$$S = G + 1 - A$$

in which

S = specific gravity of the dealcoholized wine;
G = specific gravity of the wine; and
A = specific gravity of the distillate obtained in the determination of alcohol.

From the sugar table giving percentage of sugar in terms of specific gravity, Table 3 appendix, ascertain the percentage by weight of extract in the dealcoholized wine corresponding to the value of S. Multiply the

[21] McCharles and Pitman, *Ind. Eng. Chem., Anal. Ed.* 8, 55 (1936).

figure thus obtained by the value of 8 to obtain the g. of extract in 100 cc. of wine.

By Evaporation—In *dry wines*, having an extract content of less than 3 g. per 100 cc: Evaporate 50 cc. of the sample on a water bath to a syrupy consistency in a 75 cc. flat-bottomed platinum dish, approximately 85 mm. in diameter. Heat the residue for 2–5 hours in a drying oven at the temperature of boiling water, cool in a desiccator, and weigh as soon as the dish and contents reach room temperature.

In *sweet wines:* If the extract content is between 3 and 6 g. per 100 cc., treat 25 cc. of the sample as directed above. If the extract exceeds 6 g. per hundred cc., however, the result, obtained by calculation as directed in this section, is accepted and no gravimetric determination is attempted because of the inaccurate results obtained by drying levulose at a high temperature.

Glycerol—Glycerol is generally estimated in wines by the direct weighing method. If a more accurate determination is necessary, the oxidation method used for vinegars may be applied. For sweet wines or wines whose extract exceeds 5 g. per 100 cc., the sugar is removed with milk of lime. Heat 100 cc. of the wine to boiling and add successive small portions of milk of lime until the sample becomes progressively darker and then lighter in color. Cool, add 200 cc. of 95 per cent alcohol, allow the precipitate to settle and filter. Wash the residue with 95 per cent alcohol. Evaporate the combined filtrate and washings on a water bath. Dry wines may be analyzed for glycerol by evaporating 100 cc. and continuing from this point. Proceed with the evaporation on the water bath down to a volume of 10 cc. Treat the residue with about 5 g. of fine sand and 4–5 cc. of milk of lime (a mixture containing 15 grams calcium oxide per 100 cc.) for each gram of extract, and continue the evaporation almost to dryness. Add 50 cc. of alcohol 90 per cent by volume and rub the residue to a paste, incorporating the material adhering to the sides of the dish. Heat the mixture on a low-heat hot plate to incipient boiling with constant stirring and decant the liquid through a filter into a small flask. Wash the residue repeatedly by decantation with 10 cc. portions of hot 90 per cent alcohol until the filtrate totals 150 cc. Transfer the filtrate to a porcelain dish and evaporate to a syrupy consistency on a low-heat hot plate or steam bath regulated below boiling temperature. Transfer the residue to a glass-stoppered cylinder with 20 cc. of absolute alcohol. Add 3 ten cc. portions of anhydrous ether, shaking thoroughly

after each addition. Allow to stand until clear. Filter, catching the filtrate in a tared dish. Wash the cylinder and filter with a mixture of 2 parts of absolute alcohol to 3 parts of anhydrous ether, also pouring the wash liquor through the filter. Evaporate the filtrate and washings to a syrupy consistency, dry for an hour at 100° C., weigh, ignite, weigh. The loss on ignition gives the weight of glycerol.

To determine glycerol by the oxidation method proceed with the above directions up to the point of addition of sand and milk of lime. From that stage in the directions, the method as given under the section on vinegar, Chapter XII, should be followed.

Total Acids—This term is used to describe the total titratable acidity and includes both volatile and fixed acids and is calculated as tartaric acid. Twenty cc. of the wine is placed in a flask and heated rapidly to incipient boiling. It is then immediately titrated with 0.1 *N* sodium hydroxide solution. For white wines, phenolphthalein may be used as an inside indicator and for highly colored wines, phenolphthalein powder, one part of phenolphthalein powder mixed with 100 parts of dry, powdered potassium sulfate, or 0.05 per cent azolitmin solution may be used as outside indicators with the aid of a spot plate. If the color is only moderately deep, the wine may be diluted with 50 to 150 cc. of recently boiled and cooled water. One cc. of 0.1 *N* sodium hydroxide solution = 0.0075 g. of tartaric acid.

Volatile Acids—Substances volatile with steam and titratable with alkali are termed volatile acids. This may be simply done by steam distilling 10 cc. of the sample until 50–100 cc. of distillate is collected. The distillate is titrated with 0.1 *N* sodium hydroxide solution using phenolphthalein as indicator. The volatile acids are expressed as acetic acid and 1 cc. of the 0.1 *N* sodium hydroxide solution is equivalent to 0.0060 g. of acetic acid

The volatile acids may also be determined indirectly by determining the residual acids after evaporation and subtracting the result obtained from the total acids. Evaporate 25 cc. of the sample in a porcelain dish on a water bath or a low heat hot-plate to about 3–5 cc. Redissolve the residue in 25 cc. of water and again evaporate to a volume of 3–5 cc Repeat a third time. Finally dissolve the residue in water and titrate as directed above under the section total acids. Subtract the result from that ascertained in the total acid determination. The difference equals volatile acids.

The indirect method is subject to error because of the decomposition of acid salts and because of loss of some of the less volatile acids due to the repeated evaporations. However, for many purposes, the result obtained is as useful as that derived by the longer methods.

Volatile acids may be accurately determined by use of the Sellier distillation apparatus, Fig. 53. Transfer 10 cc. of the sample free of carbon dioxide to the inner tube of the apparatus, add a small piece of paraffin to prevent foaming. Place the tube within the larger flask, which contains 100 cc. of recently boiled water. Connect the inner tube with an inclined condenser by means of a Kjeldahl trap and distill by heating the outer flask. Continue the distillation until 50 cc. has been collected in an Erlenmeyer flask by means of an adapter attached to the end of the

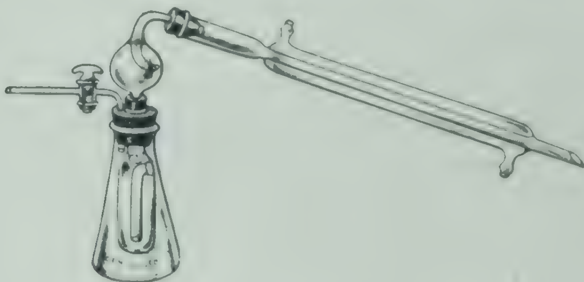

Fig. 53. Sellier Distillation Apparatus
(Courtesy of Central Scientific)

condenser. Titrate with 0.1 *N* sodium hydroxide solution, using phenolphthalein as indicator. Continue the distillation, titrating after collecting each 10 cc. until not more than 1 drop of standard alkali is required to reach the neutral point. Usually 80 cc. of distillate contains all of the volatile acids.

Fixed Acids—The result obtained by titrating the remaining acids in the indirect method for volatile acids may be expressed as fixed acids. It may also be calculated by multiplying the quantity of volatile acids by 1.25 and subtracting the result from the total acids. Fixed acids may also be determined by evaporation of 50 cc. of wine to dryness. Dry at 105° C. and then dissolve the residue and titrate in the usual manner.

Tartaric Acid—The methods of the A. O. A. C. give the following directions: Neutralize 100 cc. of the wine with *N* sodium hydroxide solution, calculating from the acidity, the number of cc. of *N* alkali necessary

for the neutralization. If the volume of the solution is increased more than 10 per cent by the addition of the alkali, evaporate to approximately 100 cc. Add to the neutralized solution 0.075 g. of tartaric acid for each cc. of N alkali added and after the tartartic acid has dissolved, add 2 cc. of glacial acetic acid and 15 g. of potassium chloride. After the potassium chloride has dissolved, add 15 cc. of 95 per cent alcohol; stir vigorously until the potassium bitartrate begins to precipitate; and let stand in an ice box for at least 15 hours at 15–18° C. Decant the liquid from the separated potassium bitartrate on a Gooch crucible prepared with a very thin film of asbestos, or on filter paper in a Büchner funnel. Wash the precipitate from the beaker with the filtrate (keep cold) and finally rinse the beaker and filter 3 times with a few cc. of a mixture of 15 g. of potassium chloride, 20 cc. of 95 per cent alcohol, and 100 cc. of water, using not more than 20 cc. of the wash solution in all. Transfer the asbestos or paper and precipitate to the beaker in which the precipitation was made; wash the Gooch crucible or Büchner funnel with hot water, using about 50 cc. in all; heat to boiling; and titrate the hot solution with 0.1 N sodium hydroxide solution using phenolphthalein indicator. Increase the number of cc. of 0.1 N sodium hydroxide required by 1.5 cc. to allow for the solubility of the precipitate. One cc. of 0.1 N sodium hydroxide alkali is equivalent, under these conditions, to 0.015 g. of tartaric acid. To obtain the g. of total tartaric acid per 100 cc. of the wine, subtract the quantity of tartaric acid added from this result.

The method is based on the precipitation of the free and combined tartaric acid as the potassium acid salt, $KHC_4H_4O_6$, which is not very soluble in the cold. The acid salt is then dissolved and titrated with the alkali solution. The acetic acid is added to decrease the solvent action of the hydrochloric acid formed in the reaction $KCl + H_2C_4H_4O_6 \rightleftharpoons KHC_4H_4O_6 + HCl$.

Free Tartaric Acid and Cream of Tartar—Free tartaric acid and cream of tartar, that is, potassium acid tartrate may be calculated in the following manner:

Let A = total tartaric acid in 100 cc. of wine, divided by 0.015;
B = total alkalinity of the ash (sum of C and D)
C = alkalinity of water-soluble ash; and
D = alkalinity of water-insoluble ash

Then

1) if A is greater than B,
Cream of tartar = $0.0188 \times C$, and
Free tartaric acid = $0.015 \times (A - B)$;

2) if $A = B$ or is smaller than B but greater than C
Cream of tartar = $0.0188 \times C$, and
Free tartaric acid = 0 and

3) if A is smaller than C
Cream of tartar = $0.0188 \times A$ and
Free tartaric acid = 0

Sorbitol—The adulteration of wine with cider or perry, pear juice, may be demonstrated by showing the presence of sorbitol which is not a natural constituent of grapes and hence of wine. Reif [22] states that it may be detected in the presence of dulcin and saccharin either by the formation of its hexacetyl compound, or by the color reaction of its benzylidene derivative with acetone in the presence of sulfuric acid. In the latter case it is the liberated benzaldehyde which gives the colored compound with the acetone. Benzaldehyde and sulfuric acid (1 : 1) act on dulcin, *p*-phenetole carbamide, giving benzylidenedulcin, with formation of a red color. Dulcin also forms a crystalline acetyl compound, and benzylidenedulcin reacts with aqueous sulfuric acid and acetone to yield benzaldehyde and a yellowish-red color. Dulcin and saccharin are completely adsorbed by charcoal. Sorbitol, on the other hand, is not adsorbed. Hence, by adsorption on charcoal dulcin and saccharin may be removed as interfering substances.

From 100 to 130 cc. of the wine are heated with 7 g. of carbon and filtered by suction through Seitz filtering material. The filtrate is placed in a 300 cc. distilling flask, carrying a capillary tube through its cork, reaching to the bottom of the flask, and closed by rubber tubing and a pinch cock. The side arm is connected with a vacuum pump, and the solution is concentrated on a water-bath under reduced pressure until it becomes strongly viscous. The vacuum is maintained until room temperature is reached, when 4 drops of benzaldehyde and 1 cc. of sulfuric acid (1 : 1) is added, and after shaking, the flask is left overnight in a refrigerator at 7° C.

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[22] Reif, *Z. Untersuch. Lebensm.* 66, 408 (1933).

If 10 per cent or more of fruit juice is present, the mass will be solid, but with pure wine it will be liquid.

The subsequent procedure is varied according to the amount of benzylidenesorbitol formed. With a relatively large amount, the residue is washed from the flask with 100 cc. of cooled water into a beaker and left for 30 minutes. It is then filtered by suction on to a fritted glass crucible (pore size 1 G2), and washed, first with about 100 cc. of water, any lumps being broken up with a glass rod, then with two 5–10 cc. portions of an ice-cold mixture of 3 parts of absolute alcohol and 7 parts of petroleum ether, and, finally, twice with at least 10 cc. of petroleum ether. Before each addition of washing liquid, the suction is interrupted so that the precipitate may be thoroughly mixed with the added liquid. The precipitate is then dried in the crucible or on a watch glass for 30–60 minutes at 75–80° C. To 0.01–0.03 g. of the dry precipitate, which should be practically colorless, in a test tube, 0.9 cc. of water and 0.3 cc. of pure acetone are added, and the whole is mixed by swirling the tube; 0.52 cc. of sulfuric acid (sp. gr. 1.84) is then pipetted rapidly on to the middle of the liquid surface, and the swirling is repeated. If benzylidenesorbitol is present, the crystals will dissolve and the acid liquid assumes, either at once or after a few minutes, an orange-red color, which reaches its full strength after about 15 minutes. If only benzylidenemannitol is present, the crystals also dissolve, but the liquid either remains colorless or turns yellow.

If less than 0.1 g. of the precipitate forms, it is best collected on a small filter paper in a Gooch crucible, washed with 20 cc. of water, two 3 cc. portions of the alcohol petroleum ether mixture, and two 10 cc. portions of petroleum ether, no interruption of the suction or stirring being necessary. After being dried for 30 minutes at about 75° C., the paper, folded with the precipitate inside, is transferred to a test tube and 0.45 cc. of water and 0.15 cc. of acetone are added. The filter is pressed into the liquid with a rod, 0.26 cc. of sulfuric acid is added, and the tube is swirled. The orange-red color may take an hour to develop. The procedure shows clearly the presence of 3 per cent of cider in wine and gives a faint pink color with 2 per cent.

If the results are doubtful, the test should be repeated with 200–300 cc. of wine and the precipitate of benzylidenesorbitol should be transformed into the easily crystallizable hexacetylated sorbitol. A blank test should be made with a wine of known purity of the same district, and also a test made with the wine of known purity to which has been added 10 per cent of cider.

Tannin and Coloring Matter—The A. O. A. C. gives the following details for the determination of tannin and coloring matter.

Reagents: (a) Oxalic acid solution. 0.1 *N* 1 cc. = 0.00416 g. of tannin.

(b) Standard potassium permanganate solution. Dissolve 1.333 g. of potassium permanganate in 1 liter of water and standardize the solution against (a) above.

(c) Indigo solution. Dissolve 6 g. of sodium indigotine disulfonate in 500 cc. of water by heating; cool, add 50 cc. of sulfuric acid, make up to a liter and filter.

(d) Purified boneblack. Boil 100 g. of finely powdered boneblack with successive portions of hydrochloric acid (1 : 3), filter, and wash with boiling water until free from chlorides. Keep covered with water.

Determination: Dealcoholize 100 cc. of the wine by evaporation and dilute with water to the original volume. Transfer 10 cc. to a 2 liter porcelain dish and add about 1 liter of water and exactly 20 cc. of the indigo solution. Add the standard potassium permanganate solution, 1 cc. at a time, until the blue color changes to green; then add a few drops at a time until the color becomes golden yellow. Designate the number of cc. of potassium permanganate solution as "a". Treat 10 cc. of the prepared dealcoholized wine for 15 minutes with boneblack, filter, and wash thoroughly with water. Add 1 liter of water and 20 cc. of the indigo solution and titrate with potassium permanganate, as directed above. Designate the number of cc. of potassium permanganate solution used as "b".

Then a − b = c, the number of cc. of the potassium permanganate solution required for the oxidation of the tannin and coloring matter in 10 cc. of the wine.

Wine Ratios

Glycerol-Alcohol Ratio: This ratio is expressed as X : 100 in which X is obtained by multiplying the percentage weight of glycerol by 100 and dividing the result by the percentage of alcohol by weight.

Ash-Extract Ratio: This ratio is expressed as 1 : X in which X is the quotient obtained by dividing the grams of extract per 100 cc. by the grams of ash per 100 cc.

Alcohol-Extract Ratio: This ratio is expressed as X : 1 in which X is the quotient obtained by dividing the percentage by weight of alcohol by the reduced extract. The reduced extract is given by the expression:

Reduced Extract $= E - [S - 0.1] - [K - 0.1]$

in which

$E =$ g. of extract
$S =$ g. of sugar per 100 cc. of wine
$K =$ g. of potassium sulfate per 100 cc. of wine.

Interpretation of Results—Wines

The alcohol content generally lies between 5–10 g. per 100 cc. of non-fortified wines. Alcohol in excess of 14.5 g. should be interpreted as fortified. The variation in alcohol content is so great that small value can be given to this constant.

The glycerol content varies between 0.4 to 1 per cent but may go as low as 0.16 or as high as 1.4 per cent. German authorities stress the glycerol-alcohol ratio. For European wines this ratio falls within the range 7 to 14, with 5 to 20 as outside limits. The American average is nearer 5.5.

French chemists place more reliance on the alcohol/extract ratio than on the glycerol-alcohol ratio. This ratio seldom exceeds 4.5 for red wines and 6.5 for white wines. However, it must be borne in mind that in the French method for the estimation of extract the glycerol is driven off. A value greater than the above may be taken as an indication of added alcohol.

The ash content of wines falls between 0.2 per cent to 0.3 per cent with outside limits of 0.11 and 0.44 per cent. Ash runs lower for white wines than for red wines. Abnormally high ash content or ash that contains more than 0.2 per cent potassium sulfate may be considered "plastered", that is gypsum has been added. Natural wines contain from 3.8 to 25 per cent of sulfur trioxide in the ash whereas plastered wines contain 40 per cent or more of sulfur trioxide.

The total acidity of wines is expressed as tartaric acid and falls between 0.4 to 1.5 per cent with 0.3 to 1.7 per cent as outside limits. In France, the sum of the alcohol content in ccs. per 100 cc. and total acidity, calculated as grams sulfuric acid per liter, is considered normal only if it exceeds 12.5. A lower value is deemed evidence of watering. When the alcohol/extract ratio exceeds the limits mentioned above, the natural percentage is substituted for that actually found. This takes care of the possible process of watering and then fortifying. For example, if a red wine contains 12 g. of alcohol and 1.5 of reduced extract (that is, the extract $-$ [% sugar $- 0.1$] $-$ [% $K_2SO_4 - 0.1$]), it is obvious that alcohol

has been added. This added alcohol must be taken into consideration in calculating added water. The reduced extract, 1.5, is multiplied by 4.5 (the average alcohol extract ratio) giving the natural percentage by weight of alcohol, and then is divided by 0.8 to obtain the percentage by volume. To the number, in this case, 8.5, the acidity in g./liter is added and if the sum is less than 12.5 it may be taken that both water and alcohol have been added.

Volatile acids, calculated as acetic usually are below 0.08 per cent with a maximum of 0.12 per cent and wine containing over 0.15 per cent should be considered unsound. It is necessary to note that in some French wines sulfites are used, obviously the sulfite content raises the volatile acids content, and correction should be made if desired.

The detection of added sucrose has been treated in one of the foregoing sections. In natural wine, the rotation is to the left, and the addition of much anhydrous dextrose would cause it to be dextrorotatory. If after inversion, a wine shows a reading to the right, dextrose or commercial glucose has been added, and if inversion changes the polarization to the left, sucrose has been added. The addition of commercial glucose may also be detected by methods previously described in the text. The addition of commercial invert sugar may give a laevorotation greater than normal. The addition of sugar solutions will tend to lower all other constants in proportion to the sugar content.

In Tables 55, 56 authentic analyses of various types of wine are given. Comparison of results obtained by analysis with these constants, plus the considerations noted above, should aid the analyst in drawing a conclusion. However, again, experience and taste are great aids in confirming analytical results.

Analysis of Beer—Beer may be analyzed as is wine. Thus specific gravity, alcohol, total acids, volatile acids, and glycerol may be estimated as detailed under the sections in wine. Ash, phosphates, sulfates, metals, preservatives, coloring matters and reducing sugars may be determined as described in other sections of the book. However, the acids are calculated to lactic acid with 0.1 *N* NaOH = 0.0090 g. of lactic acid, and reducing sugars are expressed as grams of anhydrous maltose. Extract may be determined by weighing the residue obtained from 25 cc. of the carbon dioxide-free beer by drying in a vacuum oven at 70° C. It may also be calculated as directed in the section "Extract" under wine, merely substituting the word "beer" for "wine" in the explanatory parts of the formula.

Table 55. Analyses of Wine [23]

(Receiving awards at Paris 1900)

		Sp. Gr. at 15.5°C.	Alcohol by Volume	Alcohol g./100 cc.	Glycerol g./100 cc.	Glycerol-Alcohol ratio	Extract g./100 cc.	Ash g./100 cc.	Extract—Ash ratio	Total Acids g./100 cc.
Sparkling...	max.	1.0169	15.20	12.06	0.73	....	8.56	0.29	1:8.5	0.78
	min.	0.9910	11.65	9.25	0.23	....	1.78	0.11	1:30	0.60
	ave.	1.0045	13.22	....	0.41	....	5.40	0.15	....	0.66
Dry White .	max.	0.9939	14.25	11.31	1.01	9.2:100	2.51	0.27	1:6.7	0.72
	min.	0.9901	10.60	8.41	0.57	5.7:100	1.55	0.17	1:16.4	0.43
	ave.	0.9917	12.45	....	0.70	....	1.99	0.20	....	0.59
Dry Red...	max.	0.9969	15.40	12.22	0.95	8.1:100	3.22	0.39	1:6.5	0.90
	min.	0.9926	10.10	8.01	0.59	5.8:100	1.77	0.14	1:18.6	0.45
	ave.	0.9943	12.61	....	0.64	....	2.57	0.25	....	0.65
Sweet White	max.	1.0494	21.55	17.10	0.59	....	19.35	0.26	1:4.5	0.81
	min.	0.9908	11.60	9.21	0.13	....	5.71	0.10	1:28.7	0.16
	ave.	1.0298	18.38	....	0.30	....	13.80	0.20	....	0.41
Sweet Red..	max.	1.0522	22.05	17.50	0.75	....	19.71	0.37	1:8.4	0.83
	min.	1.0107	13.70	10.87	0.29	....	7.57	0.23	1:16.6	0.40
	ave.	1.0276	19.30	....	0.51	....	13.52	0.31	....	0.50

[23] Wiley, U. S. Dept. Agr., Bur. Chem., Bull. No. 72 (1903).

TABLE 55. ANALYSES OF WINE[23] (*Continued*)

(Receiving awards at Paris 1900)

		Fixed Acids g./100 cc.	Volatile Acids g./100 cc.	Total Tartaric Acid g./100 cc.	Free Tartaric Acid, g./100 cc.	Volatile Acid –Total Acid Ratio	Reducing Sugars g./100 cc.	Potassium Sulfate g./100 cc.	Total Sulfurous Acid, g./100 cc.	Tannin, g./100 cc.
Sparkling	max.	0.72	0.15	0.36	0.14	1:4.07	5.23	0.05	0.045	0.07
	min.	0.42	0.05	0.16	0.00	1:14.5	0.02	0.00	0.001	0.009
	ave.	0.57	0.08	0.27	0.07	....	3.41	0.03	0.007	0.04
Dry White	max.	0.57	0.17	0.35	0.18	1:3.83	0.33	0.13	0.016	0.06
	min.	0.33	0.05	0.06	0.00	1:9.88	0.05	0.03	0.001	0.02
	ave.	0.46	0.10	0.19	0.07	....	0.13	0.09	0.006	0.04
Dry Red	max.	0.63	0.27	0.25	0.05	1:3.38	0.28	0.13	....	0.34
	min.	0.35	0.07	0.08	....	1:7.90	0.05	0.02	....	0.14
	ave.	0.51	0.13	0.16	....	....	0.15	0.07	....	0.23
Sweet White	max.	0.53	0.22	0.30	0.14	1:2.96	16.91	0.09	0.005	0.07
	min.	0.12	0.03	0.04	0.02	1:9.47	3.71	0.02	0.001	0.02
	ave.	0.30	0.09	0.14	....	....	11.30	0.04	0.005	0.04
Sweet Red	max.	0.51	0.26	0.15	....	1:3.24	16.96	0.06	0.005	0.22
	min.	0.26	0.08	0.03	....	1:5.78	3.24	0.02	....	0.05
	ave.	0.47	0.12	0.08	....	....	10.26	0.05	....	0.10

23 Wiley, U. S. Dept. Agr., Bur. Chem., Bull. No. 72 (1903).

Table 56. Composition of Wine [24]

	Dry White	Dry Red	Sherry Material	Port
Sp. Gr. 20°/20°C	0.9919	0.9951	0.9893	1.0229
Composition, g./100 cc. wine:				
Alcohol % by volume	12.15	13.62	21.59	21.61
Volatile acid	0.051	0.048	0.030	0.021
Total acid	0.418	0.600	0.473	0.413
Extract	1.96	3.22	4.11	12.65
Total nitrogen	0.030	0.050	0.029	0.034
Reducing sugars as dextrose	0.10	0.228	2.03	9.60
Tannin and coloring matter	....	0.198	....	0.147
Ash	0.418	0.510	0.301	0.347
Alkalinity of ash[25]				
Water soluble	28.7	25.3	20.3	18.5
Water insoluble	13.5	13.5	9.6	15.5
Total tartaric acid	0.269	0.272	0.178	0.199
Potassium acid tartrate	0.337	0.341	0.223	0.250
Refractive index at 20°C	1.3419	1.3457	1.3515	1.3632
pH at 25°C	3.86	3.71	3.70	3.74
Freezing point				
°C	—5.6	—6.7	—11.7	—13.9
°F	22.	20.	10.	7.

REFERENCES

Gauging Manual, U. S. Treasury Dept. (1934).

Wiley, "Beverages and Their Adulteration," Blakiston (1919).

Allen's Commercial Organic Analysis, 5th ed., Vol. 1, Blakiston (1923).

Pharmacopoeia of the United States XI and X Decennial Revision.

Methods of the A. O. A. C.

Cox, "Analysis of Foods," Blakiston (1926).

Woodman, "Food Analysis," McGraw-Hill (1931).

Leach-Winton, "Food Inspection and Analysis," Wiley (1920).

Cruess, Joslyn and Saywell, "Laboratory Examination of Wines," Avi (1934).

Treasury Dept., "Formulae for Completely and Specially Denatured Alcohol," Appendix to Regulations, No. 61 (1926).

[24] Marsh and Joslyn, *Ind. Eng. Chem.* 27, 1252 (1935).

[25] Cc. of 0.1 *N* sodium hydroxide per 100 cc. wine.

CHAPTER XV

MEAT, MEAT PRODUCTS, FISH AND EGGS

It is seldom necessary for the food analyst to analyze fresh meat as such except for investigational work. However, other occasions do arise when such analyses must be made. Meat is the properly dressed flesh (the edible part of the striated muscle of an animal) derived from cattle, from swine, from sheep, or from goats, sufficiently mature and in good health at the time of slaughter, but restricted to that part of the striated muscle which is skeletal or that which is found in the tongue, in the diaphragm, in the heart, or in the esophagus, and does not include that found in the lips, in the snout, or in the ears, with or without the accompanying and overlying fat, and the portions of bone, skin, sinew, nerve, and blood vessels which normally accompany the flesh, and which may not have been separated from it in the process of dressing it for sale. *Beef* is the meat derived from cattle nearly one year of age or older. *Veal* is the meat derived from young cattle one year or less of age. Veal genrally implies meat from an immature milk-fed bovine animal usually not over three months of age. *Calf*, on the other hand, implies meat from an immature bovine animal which for a considerable time had subsisted in part or entirely on feeds other than milk. *Mutton* is meat derived from sheep nearly one year of age or older. *Lamb* is the meat derived from young sheep one year or less of age. *Pork* is the meat derived from swine.

In general, the food analyst has occasion to analyze prepared meats, for these products are subject often to a wide variety of forms of adulteration, or as some authorities might say, substitution. The excessive addition of water, cereal, starch and soya flour are examples of adulteration and substitution in such products as smoked meats, sausage and potted meat.

It is not only instructive, but an aid, in judging the analyses to be made on a sample, to bear in mind the definitions of meat products as set by the Food and Drug Administration, U. S. Deparment of Agriculture.

Prepared meat is the product obtained by subjecting meat to a process

of comminuting, of drying, of curing, of smoking, of cooking, of seasoning, or of flavoring, or to any combination of such processes.

Cured meat is the product obtained by subjecting meat to a process of salting, by the employment of dry common salt or of brine, with or without the use of one or more of the following: Sodium nitrite, sodium nitrate, potassium nitrate, sugar, dextrose, a syrup, honey, spice.

Dry salt meat is the prepared meat, which has been cured by the application of dry common salt, with or without the use of one or more of the following: Sodium nitrite, sodium nitrate, potassium nitrate, sugar, dextrose, a syrup, honey, spice; with or without the injection into it of a solution of common salt to which may have been added one or more of the following: Sodium nitrite, sodium nitrate, potassium nitrate, sugar, dextrose, a syrup, honey.

Corned meat is the prepared meat which has been cured by soaking in, with or without injecting into it, a solution of common salt, with or without one or more of the following, each in its proper proportion: Sodium nitrite, sodium nitrate, potassium nitrate, sugar, dextrose, a syrup, honey, and with or without the use of spice.

Sweet pickled meat is the prepared meat which has been cured by soaking in, with or without injecting into it a solution of common salt, with sugar and/or dextrose, a syrup, and/or honey, together with one or more of the following, each in its proper proportion: Sodium nitrite, sodium nitrate, potassium nitrate, and with or without the use of spice.

Dried meat is the product obtained by subjecting fresh meat or cured meat to a process of drying, with or without the aid of artificial heat, until a substantial portion of the water has been removed.

Smoked meat is the product obtained by subjecting fresh meat, dried meat, or cured meat to the direct action of the smoke either of burning wood or of similar burning material.

Canned meat is fresh meat or prepared meat, packed in hermetically sealed containers, with or without subsequent heating for the purpose of sterilization.

Hamburg steak or hamburger steak is comminuted fresh beef, with or without addition of suet and/or of seasoning.

Potted meat or deviled meat is the product obtained by comminuting and cooking fresh meat and/or prepared meat, with or without spice. It is usually packed in hermetically sealed containers.

Sausage meat is fresh meat or prepared meat, or a mixture of fresh meat and prepared meat. It is sometimes comminuted. The term

"sausage meat" is sometimes applied to bulk sausage containing no meat byproducts.

Meat byproducts are any properly dressed edible parts, other than meat, which have been derived from one or more carcasses of cattle, of swine, of sheep or of goats, sufficiently mature and in good health at the time of slaughter.

Meat food products are any articles of food or any articles that enter into the composition of food which are not prepared meats but which are derived or prepared, in whole or in part, by a process of manufacture from any portion of the carcasses of cattle, swine, sheep, or goats, if such manufactured portion be all, or a considerable and definite portion, of the article, except such preparations as are for medicinal purposes only.

Meat loaf is the product consisting of a mixture of comminuted meat with spice and/or with cereals, with or without milk and/or eggs, pressed into the form of a loaf and cooked.

Pork sausage is chopped or ground fresh pork, with or without one or more of the following: Herbs, spice, common salt, sugar, dextrose, a syrup, water.

Brawn is the product made from chopped or ground and cooked edible parts of swine, chiefly from the head, feet, and/or legs, with or without the chopped or ground tongue.

Headcheese, or mock brawn is the product made from chopped or ground, cooked edible parts of meat or meat byproducts.

Souse is the product consisting of meat and/or meat byproducts; after cooking, the mixture is commonly packed into containers and covered with vinegar.

Scrapple is the product consisting of meat and/or meat byproducts mixed with meal or the flour of grain, and cooked with seasoning materials, after which it is poured into a mold.

Frankfurter and *bologna style sausage* are important meat products. The following definitions, although not those of the Food and Drug Administration, are entirely descriptive. Frankfurter style sausage of the best quality is prepared from meat, usually a mixture of beef and pork trimmings and certain beef cuts.[1] The ground mixture is stuffed in animal or artificial casings and is then smoked and cooked for a short time. Cheaper grades of frankfurter style sausage are made of a mixture of meat and meat byproducts. Cereal is frequently added to these grades of sausage.

[1] Hoagland, U. S. Dept. Agr. Circ. No. 230 (1932).

Bologna style sausage is prepared from ingredients similar to those used in frankfurter style sausage and in a similar manner, except that the former is stuffed in larger casings or containers and is cooked for a longer time. The cheaper grades of this product are made from much the same materials as those used for the same grades of frankfurter style sausage. Kosher bologna style sausage and kosher frankfurter style sausage contain no pork.

Composition of Meat

The constituents of the muscle of any animal vary between certain limits just as the constituents of milk, blood, urine, or any body fluid or section vary between certain limits, and as we set the term constants for the percentage of these components, variations in these constants may be used for the detection of adulteration and sophistication.

The muscle matter of animals generally contains about 75 per cent water and 25 per cent total solids. The total solids consist of about 20 per cent nitrogenous and non-nitrogenous material and extractives, and mineral salts or ash. The remaining 80 per cent is protein material. Among the important nitrogen extractives are creatine, creatinine, uric acid, urea, and the xanthine bodies or purine bases, xanthine, guanine and carnine. Among the important non-nitrogenous extractives are lactic acid, glycogen and fatty materials. The moisture content of lean meat tends to approach 80 per cent as a maximum. Lean meat consists essentially of muscle tissue, connective tissue and intramuscular fat cells, the percentage of the latter two components being small.

Tables 57, 58, 59, 60 and 61 give the percentage composition of lean and prepared meats. It can be seen from the preceding paragraph and from Table 62, that in lean normal meat the moisture-protein ratio tends to become 4:1 as a maximum with the more usual ratio as 3.5:1. As meat is processed, treated or prepared in any way whatever, it tends to lose moisture unless this loss is deliberately counteracted. Hence the tendency of manufacturers and processors is to attempt to equalize the loss by the incorporation of water. At times this incorporation is carried to fraudulent lengths. As meat or meat products stand they also tend to lose moisture. In order to prevent the excessive loss of moisture in such products as sausage, it has become customary to incorporate not only a small portion of water, as ice, which aids in the manufacturing process, but also binders such as cereal, starch, soy bean flour, skim milk powder and other materials. At times the addition of these materials assumes deceitful dimensions.

TABLE 57. COMPOSITION OF FRESH LEAN BEEF

Type	Moisture %	Protein %	Fat %	Ash %
Ox	76.7	20.0	1.5	1.2[2]
Ox	76.71	20.78	1.5	1.18[2]
Cow	76.35	20.54	1.78	1.32[2]
Medium Ox	73.7	19.8	5.3	1.2[2]
Chuck	71.3	20.2	8.2	1.0[3]
Chuck	72.84	19.81	7.13[2]	....
Cattle, fat free	76.5	21.88	....	1.1[2]
Cattle	76.11	....	....	....
Cattle, emaciated	80.09	18.90	0.45	0.99[2]
Cattle, thin	78.84	19.65	0.75	1.03[2]
Very thin	74.24	20.25	2.78	0.93[2]
Thin	73.52	19.81	4.91	1.00[2]
Very very thin	76.37	18.94	1.87	1.05[2]
Thin cow	68.70	20.00	9.52	0.98[2]
Chuck, very lean	73.8	22.3	3.9	1.0[3]
Chuck rib	75.8	22.2	1.4	1.1[3]
Chuck rib	71.3	19.5	8.3	0.8[3]
Chuck	74.1	22.6	2.8	1.1[3]
Flank	70.7	25.9	3.3	1.2[3]

TABLE 58. COMPOSITION OF PREPARED BEEF

Type	Moisture %	Protein %	Fat %	Ash %
Boiled	51.8	25.5	22.5	1.3[4]
Corned canned	51.8	26.3	18.7	4.0[4]
Dried canned	44.8	39.2	5.4	11.2[4]
Roast canned	58.9	25.9	14.8	1.3[4]
Luncheon canned	52.9	27.6	15.9	4.8[4]
Corned	53.6	15.6	26.2	4.9[4]
Spiced and rolled	30.0	12.0	51.4	6.8[4]
Roast cooked	48.2	22.3	28.6	1.3[4]
Round steak	63.0	27.6	7.7	1.8[4]
Sandwich meat	58.3	28.3	11.0	2.8[4]
Loin steak	54.8	23.5	20.4	1.2[4]
Corned	63.86	26.01	8.06	2.80[5] (salt)

[2] Allen's Commercial Organic Analysis, Vol. IX, 5th ed., Blakiston (1932).

[3] Atwater and Bryant, U. S. Dept. Agr., Bull. No. 28 (1906).

[4] Atwater and Bryant, U. S. Dept. Agr., Bull. No. 28 (1906).

[5] Eakins, "Military Meat and Dairy Hygiene," p. 317, Williams and Wilkins (1924).

Analysis for the percentage of the constituents of a meat or meat product and comparison with the normal analyses shown in Tables 57 to 61 aid in the interpretation of whether a processed, prepared or treated meat or meat product has been manufactured along normal lines, or whether it has been adulterated.

TABLE 59. COMPOSITION OF FRESH AND PREPARED BEEF TONGUE

Type	Moisture %	Protein %	Fat %	Ash %
Fresh	70.8	18.9	9.2	1.0[6]
Canned, ground	49.9	21.4	25.1	4.0[6]
Canned, whole	51.3	19.5	23.2	4.0[6]
Pickled	62.3	12.8	20.5	4.7[6]
Fresh, ox	68.3	18.13	11.46	1.18[7]
Fresh	64.00	15.20	19.15	0.80[7]
Potted	41.52	18.46	32.85	6.7[8]

TABLE 60. COMPOSITION OF FRESH AND PREPARED HAM

Type	Moisture %	Protein %	Fat %	Ash %
Fresh lean	60.0	25.0	14.4	1.3[9]
Smoked lean	53.5	19.8	20.8	5.5[9]
Cooked	49.2	22.5	21.0	5.8[9]
Deviled	44.1	19.0	34.1	3.3[9]
Visible fat removed	64.5	19.2	16.2	0.9[9]
Smoked, boiled	51.3	20.2	22.4	6.1[9]
Fresh	51.55	15.29	32.37	0.8[10]
Cured	64.61	21.06	13.6	...[10]

6 Atwater and Bryant, U. S. Dept. Agr., Bull. No. 28 (1906).

7 Wright and Forsyth, *J. Soc. Chem. Ind.* **46,** 36T (1927).

8 König, "Chemie der Menschlichen Nahrungs und Genussmittel," Springer (1898).

9 Atwater and Bryant, U. S. Dept. Agr., Bull. No. 28 (1906).

10 Allen's Commercial Organic Analysis, Vol. IX, 5th ed., Blakiston (1932).

Table 61. Average Chemical Composition of Sausage and Other Meat Food Products [11]

Product	Water %	Ash %	Fat %	Protein %	Starch %
1st grade frankfurter style	60.88	2.64	22.00	13.69	....
2nd grade frankfurter style	61.33	2.95	19.70	14.51	....
Frankfurter style and cereal	64.29	3.12	14.06	15.24	1.20
1st grade bologna style	63.98	2.82	18.11	14.35	....
2nd grade bologna style	64.03	3.16	17.34	14.48	....
Bologna style with cereal	62.38	3.31	15.92	14.78	1.81
Pure pork	41.93	2.09	44.83	10.81	....
Fresh link	44.75	2.48	41.17	11.28	....
Braunschweiger style	56.20	2.67	23.75	15.39	....
Liver sausage	58.97	2.23	20.57	16.69	....
Headcheese	62.04	2.23	20.26	15.04	....
Blood sausage	47.09	2.30	34.64	14.81	....
Meat loaf	63.99	3.52	13.45	16.14	....
Souse	72.87	1.87	12.34	13.18	....
Luncheon roll	56.43	3.39	23.79	15.89	....
Polish style	56.04	3.55	23.06	16.41	....
Country style	51.74	3.92	27.45	16.21	....
Bockwurst	63.53	2.43	21.85	11.70	....

Table 62. Water-Protein Ratio of Lean Meat

Type	Ratio
Pork[12]	3 : 1
Beef[12]	3.26 : 1
Mixed meat[12]	3.13 : 1
Composite lean pork[13] (118)	3.4 : 1
Composite lean beef[13] (34)	3.6 : 1
Fresh pork[14]	3.4 : 1
Fresh beef[14]	3.6 : 1
Mixed meat[14]	3.5 : 1

Preparation of Sample

In the analysis of meat and meat products, it is usual to remove the skin, bones, and visible fat and to perform the analysis on the lean meat. This portion of the meat is passed through a meat chopper at least 3 times and if any water separates out it is reincorporated. The mixed

[11] Hoagland, U. S. Dept. Agr., Circ. No. 230 (1932).

[12] Stubbs and More, *Analyst* **44**, 125 (1919).

[13] Moulton, "Meat Through the Microscope," Chicago (1929).

[14] Jackson and Jones, *Analyst* **57**, 562 (1932).

meat is then kept in hermetically sealed containers, until needed for the analysis or the entire sample may be dried, reground and then kept in containers until the analysis may be performed. If it is desired to make the analysis on the entire sample, the bones are separated and pulverized and then the entire mass, skin, bones and meat passed through the meat chopper until the mass is uniform in composition. Or the entire sample may be dried and then ground and reground until a homogeneous mass is obtained. In the case of sausages, the outer wrapper is removed and the interior is then prepared as above.

Moisture

The moisture content of meat and meat products may be estimated simply by the direct heat method. Approximately 5 g. of the prepared sample is accurately weighed into a tared low, flat-bottomed dish and placed in an oven thermostatically controlled at 75°–80° C. for 24 hours. At the end of this period, the dish is removed, placed in a desiccator, allowed to cool and weighed. The loss in weight is calculated as moisture. This determination should be done in triplicate, for some determinations, yielding low results because of the inclusion of unevenly distributed cartilage, should be discarded.

The moisture content may also be determined by the toluene distillation method described in Chapter I. For very accurate results, a weighed portion of the sample should be dried over sulfuric acid.

Protein

At the same time that the moisture determination is performed, if it be found convenient, approximately 2 g. of the sample may be accurately weighed on a piece of filter paper on a watch glass counterpoised by another watch glass and filter paper. Transfer the filter paper and meat to an 800 cc. Kjeldahl flask and proceed to determine the albuminoid nitrogen as directed in the Kjeldahl-Gunning-Arnold method in Chapter I. Run a blank as directed and include the filter paper. The protein content is estimated by multiplying the nitrogen content by the factor 6.25.

If the residue from the moisture determination is practically fat free, the residue may be ground in a mortar and the protein determination may be made on a 0.7 g. portion of the dried and ground material. Of

course, the same procedure is to be followed if the original sample was dried and ground.

The Farinacci method may be used to determine the nitrogen content on correspondingly smaller portions of the meat sample. These determinations may also be made on the residue from the ether extraction and corrections are mathematically applied for the determinations made on the moisture and fat free basis.

Added Water

Added water may be calculated in meat or a meat product in which the accepted moisture-protein ratio is 4:1 by use of the following formula.

$$W - 4\,P = \text{added water.}$$
$$W = \text{moisture content.}$$
$$P = \text{protein content.}$$

The determination of added water in meat and meat products is becoming an estimation of increasing importance. For many years it has been customary to inject hams with the pickling solution near the bone to prevent bone and shank sour. This method of intramuscular injection aided in promoting a surer cure. Recently a method of intravenous injection or pumping has been developed and used in the processing of beef tongues. In corned beef, also, the intramuscular method has been applied. The original object of the injection or pump method of cure was to provide a faster and at the same time a surer cure for without prior injection, the meat had to be soaked for long periods before it was completely cured. This vat method of soaking is known as the "dry" cure method.

The ability of the meat to retain water thus injected into it is very high and consequently unscrupulous manufacturers and dealers have used the pump cure method to increase excessively the weight of the cured meat. This type of adulteration may easily be determined by means of estimation of the moisture and protein content.

Not only have brine and pickling solutions been injected but also solutions of gelatin. This problem and that of the injection of viscolized fats will be discussed subsequently.

In the manufacture of sausage, the meat loses moisture due to heat developed by the friction of the knives and due to other causes. This is counteracted by the processor by the inclusion of chopped ice in the comminuting step in the preparation of the sausage. Some authorities

feel that it is very difficult to prepare a succulent sausage without the addition of water. At any rate, governmental agencies have held that the addition of more than 3 per cent of moisture to uncooked sausage and of 10 per cent of moisture in smoked or cooked sausage is an adulteration. Added water may be estimated in an analogous manner to that detailed above.

Since binders are permitted in the manufacture of sausage the addition of moisture and its estimation becomes far more difficult than the simple procedure outlined in the case of tongue, corned beef and ham. Many of the binders permitted are protein bearing materials, as for example, skim milk powder and soy bean flour. In the case of skim milk powder the casein content may be determined with a fair degree of accuracy as detailed in Chapter VII, section, "The Detection of Milk Powder in Foods." The total protein found should then be corrected for the casein content. Methods for the detection and estimation of soy bean flour will be discussed subsequently. Corrections for the amount of soy bean flour protein present should be applied in calculating the added water.

Other factors to bear in mind are that the nitrogen thus determined comes not only from the protein material in the meat and meat product but also from the nitrogenous extractives such as creatine and creatinine. However, the inclusion of nitrogen from such sources acts to increase the apparent protein content.

Fat

At times it is of value and necessary to estimate the fat content of meat. A rapid, though only fairly accurate method that may be used is a modified Gerber method. Two and one-half g. of the meat is weighed into a scoop described in the Gerber method for soft curd cheese. The scoop and its contents is then placed into a cheese butyrometer and 9 cc. of 4 per cent borax solution is added and the mixture is heated in a water bath until the meat is softened. One cc. of amyl alcohol is added and then 10 cc. of the acid (to 13 parts water add 87 parts sulfuric acid, sp. gr. 1.82), used for ice cream determinations added cautiously. The butyrometer is stoppered and then shaken vigorously. When the meat has been thoroughly disintegrated by the acid, centrifuge for the required 10 minutes. The butyrometer is then removed from the centrifuge and read in the usual manner. If any small particles of meat have not been dissolved by the acid, the butyrometer may be placed in a boiling water

bath and shaken at intervals until all the meat is dissolved. The butyrometer is then recentrifuged and the fat read after removal from the centrifuge. This method will give results that are accurate within the limits of the readability of the butyrometer.

An alternative method is to weigh 1 g. of the properly prepared sample into a Jacobs-Singer separatory flask or into a tall form 100 cc. beaker and add 9 cc. of water and 1 cc. of ammonium hydroxide. Warm on a hot plate, stirring with a glass rod or by shaking until the meat is thoroughly softened. Add ½ cc. of hydrochloric acid and stir. Add 10 cc. more of hydrochloric acid and a pinch of sand. If a beaker was used, cover the beaker with a watch glass on glass hooks and boil the mixture in the flask or beaker gently for 5 minutes or until the meat is completely dissolved. Cool the mixture and transfer if a beaker was used to a Mojonnier extraction tube or a Jacobs-Singer separatory flask. Rinse the tall form beaker with sufficient water to bring the level of the water up to the middle of the constriction in the Mojonnier extraction tube or the flask and then rinse the beaker, watch glass and glass hooks with 25 cc. of ethyl ether in small portions and add the washings to the Mojonnier tube. Stopper the tube and shake vigorously. Repeat the washings and the shaking with a 25 cc. portion of petroleum ether. Allow the layers to separate and draw off the ether layer into a tared fat flask. From this point proceed as directed in the Roese-Gottlieb method for the determination of fat from the point of drawing off the ether layer into a tared fat flask.

The percentage fat or ether extract may also be determined and far more accurately by continuous extraction. Transfer the residue from the moisture determination to an extraction shell or alundum thimble. If the moisture determination was done in a soft metal dish or thin metal dish such as lead or aluminium, the dish may be cut and then entire dish and its contents placed in the extraction shell or alundum thimble. The thimble is then placed in a Soxhlet or other type continuous extractor and extracted for 16–24 hours with petroleum ether. The ether in the weighed receiving flask is evaporated and the extract dried to constant weight in an oven at 100–105° C. In order to make certain that the extraction is complete the extraction may be stopped in the middle of the extraction period, the contents removed from the thimble, ground, replaced in the thimble and the extraction with the petroleum continued to completion. The gain in weight in the receiving flask may be calculated as fat or ether extract. This process not only includes the fat but all ether soluble material.

Ash

Ash determinations may be made in the usual manner. These determinations should be made in porcelain or silica dishes because repeated ashing of meat in platinum dishes ruins the platinum. The ashing should be done below or at a dull red heat. The charred mass may be leached, filtered, the filtrate evaporated in the original dish and then the filter paper and residue ashed in the original dish.

Cold Water Extract

At times it is necessary to separate the nitrogenous constituents and obtain an idea of their proportion in the meat or meat product. This is especially important in estimating added gelatin and in detecting the addition of material that consists of nitrogenous material with only a small portion water soluble. In general a weighed portion of meat is exhausted by treating with successive portions of cold water. These extracts are filtered, made to a definite volume and portions of the filtrate are then analyzed according to the usual methods.

The A. O. A. C. recommends that 7–25 g. of the sample be weighed into a 150 cc. beaker. Fifty cc. of water is added and the mixture stirred during a 15 minute interval. The extract is filtered into a 500 cc. volumetric flask. Three successive 50 cc. portions of water, followed by four 25 cc. portions are then used. The meat is then transferred to the filter and washed finally with three 10 cc. portions of water. The filtrate is then made to the mark and thoroughly mixed. Portions of the filtrate are then used for the subsequent determinations.

Total solids of the cold water extract are determined by evaporating 100 cc. of the extract to dryness and then drying to constant weight in an oven. If the evaporation is performed in a porcelain or silica dish, the ash may be estimated by igniting the residue. Total nitrogen is determined by the Kjeldahl-Gunning-Arnold method on a 50 cc. portion of the extract.

Coagulable Proteins

One hundred and fifty cc. of the extract is evaporated in a beaker to 40 cc. on a steam bath. It is made neutral to phenolphthalein. Add 1 cc. of 0.1 N acetic acid and boil the mixture for 5 minutes. Filter through quantitative paper and transfer all of the coagulum to the filter with the aid of hot water and a rubber policeman. Wash 3

times more on the filter. Make the filtrate up to definite volume and reserve for the determination of proteose, peptone and gelatin, and creatine. Transfer the filter paper and the coagulum to an 800 cc. Kjeldahl flask and proceed with the nitrogen determination as previously detailed except that the original beaker is washed with the concentrated sulfuric acid before the acid is added to the digestion flask. This is done in order to dissolve any of the coagulated material not transferred to the filter.

Proteoses

The filtrate from the coagulable protein determination is evaporated to 30 cc. Cool, add 1 cc. of 50 per cent sulfuric acid and saturate with zinc sulfate. Heat on a steam bath with stirring until clear, allow to stand for 12 hours and filter. Wash the precipitate thoroughly with saturated zinc sulfate solution slightly acid with sulfuric acid. Determine nitrogen in the precipitate in the usual manner.

Proteose, Peptone, and Gelatin Nitrogen

Transfer a 50 cc. aliquot of the filtrate obtained from the coagulable nitrogen determination to a 100 cc. volumetric flask, add 15 g. of sodium chloride and 10 cc. of cold water, shake until the sodium chloride has dissolved, and cool to 12° C. Add 30 cc. of 24 per cent tannic acid solution cooled to 12° C., dilute to the mark with water previously cooled to 12° C., shake, and allow the mixture to stand at a temperature of 12° C. for 12 hours, or overnight. Filter at 12° C., transfer 50 cc. of the filtrate to a Kjeldahl flask, and add a few drops of sulfuric acid. Place the flask in a steam bath, connect with a vacuum pump, and evaporate to dryness. Determine nitrogen in the residue as directed in the Kjeldahl-Gunning-Arnold method. Conduct a blank determination, using the same quantity of reagent, and correct the result accordingly. Multiply the corrected result by 2 and deduct the quantity of nitrogen found from the nitrogen determined in another 50 cc. aliquot of the filtrate from the coagulable nitrogen determination without the tannin-salt treatment; the difference × 6.25 = the percentage of proteose, peptone, and gelatin.

Creatine, Folin Method

Evaporate an aliquot or the remaining portion of the filtrate and washings from the coagulable nitrogen to 5–10 cc. Transfer with a

minimum quantity of hot water to a 50 cc. volumetric flask, keeping the volume below 30 cc.; add 10 cc. of 2 N hydrochloric acid and mix. Hydrolyze in an autoclave at 117°–120° C. for 20 minutes, allow the flask to cool somewhat, remove, and chill under running water. Partially neutralize the excess of acid by adding 7.5 cc. of 10 per cent sodium hydroxide solution, free from carbonates, dilute to the mark, and mix. Make a preliminary reading on 20 cc. with a Duboscq colorimeter to ascertain the volume to use to obtain a reading of approximately 8 mm. Transfer such a volume of the solution to a 500 cc. volumetric flask and add 10 cc. of 10 per cent sodium hydroxide solution and 30 cc. of saturated picric acid solution. Mix, rotate for 30 seconds, and let stand exactly 4.5 minutes. Dilute to the mark at once with water. Shake thoroughly, and read in a Duboscq colorimeter set at 8 mm., comparing the color with 0.5 N potassium dichromate.

If the reading is too high or too low (above 9.5 or below 7 mm.), calculate the quantity necessary to obtain a reading of about 8 mm. The strength of the dichromate solution used must be checked against a standard creatine solution. Divide 81 by the reading and multiply by the volume factor to obtain the mg. of creatinine. Multiply the value obtained by 1.16 to obtain creatine, which divided by the weight of the sample and multiplied by 100 gives the percentage of creatine.

Example: Twenty g. of meat is extracted with water as directed under cold water extract and the extract is diluted to 500 cc. Treat 150 cc. of this latter solution which is equivalent to 6 g. of meat as directed in the section coagulable nitrogen. The filtrate thus obtained is then evaporated and hydrolyzed as above and diluted to 50 cc. Treat 25 cc. of this last solution with sodium hydroxide solution and picric acid solution as directed above and dilute to 500 cc. This latter solution gives a Duboscq reading of 9 mm.

$$\frac{81}{9} \times \frac{50}{25} = \text{mg. of creatinine}; \quad \frac{0.018 \times 1.16 \times 100}{6} = 0.35\% \text{ creatine.}$$

Meat Bases

Deduct from the percentage of total nitrogen, the sum of the percentages of nitrogen obtained in the determination of insoluble nitrogen, coagulable nitrogen and proteose, peptone, and gelatin, to obtain the percentage of nitrogen of the meat bases. Multiply the result by 3.12 to obtain the percentage of meat bases.

Non-Protein Nitrogen

Mezincescu and Szabo[15] suggest a method for the determination of the non-protein nitrogen in tissue that is based on estimation of the nitrogen in a trichloroacetic acid extract. About 100 g. of sample are passed through a small meat grinder. The ground tissue is thoroughly mixed with the addition of water if necessary until it is transformed into a more or less homogeneous paste, and its dry weight determined by heating a sample at 105° C. If water is added to the sample, the water content of the sample is used to calculate the dry weight and the non-protein nitrogen is calculated from the figure corresponding to the dry weight.

Three to 7 g. of the paste is weighed into a glass stoppered cylinder of about 120 cc. capacity. Add 10–20 glass beads and about 50 cc. of water; the contents are shaken for 10 minutes and then allowed to stand for 30 minutes. At the end of this time 50 cc. of 20 per cent trichloroacetic acid solution is added and the cylinder is shaken again for 10 minutes. It is left for 3 hours in a refrigerator and then its contents are filtered. The filtrate should be perfectly clear, the first portions being refiltered if necessary. Nitrogen is determined in an aliquot of the filtrate and the percentage is recalculated to the dry weight of the sample, or to the fresh weight of the sample.

Starch

This method for the estimation of starch in meat products depends on the solution of the protein, other nitrogenous, fatty, and salt constituents of meat in alcoholic potash, in which starch and materials of a like nature are insoluble. These materials are separated by filtration and then hydrolyzed to dextrose by sulfuric acid. The dextrose thus formed is estimated by the usual methods.

Any starch present in meat products generally comes from cereal or flour. Actually not only is the starch estimated but all materials of a carbohydrate nature, such as gums, insoluble in alcohol and capable of being hydrolyzed to dextrose or other reducing sugar are included in the final result. Thus, for example, although soy bean flour has practically no starch, its other carbohydrate content yields reducing sugars when subjected to this method.

Determination. Weigh 10 g. of the finely divided meat into a 250 cc.

[15] Mezincescu and Szabo, *J. Biol. Chem.* **115**, 131 (1936).

beaker. Add 75 cc. of an 8 per cent alcoholic solution of potassium hydroxide and heat on a steam or water bath until all the meat is dissolved. This generally requires 30–45 minutes. Add an equal volume of 95 per cent alcohol, cool, and allow to stand for at least an hour. Filter through a thin layer of asbestos in a Gooch crucible. Wash twice with a warm 4 per cent alcoholic solution of potassium hydroxide, 50 per cent by volume, and then twice with warm 50 per cent alcohol. Discard the washings. Retain as much of the precipitate in the beaker as possible until the last washing. Place the crucible with its contents in the original beaker and add 40 cc. of water and 25 cc. of sulfuric acid. Stir during the addition of the acid and make sure that the acid comes in contact with all the precipitate. Allow to stand about 5 minutes, add 40 cc. of water, heat just to boiling while stirring constantly. Transfer the solution to a 250 cc. volumetric flask, add 2 cc. of 20 per cent phosphotungstic acid solution, allow to cool to room temperature, and make up to the mark with water. Filter through a starch-free filter paper, pipette 100 cc. of the filtrate into a 200 cc. volumetric flask, neutralize with 20 per cent sodium hydroxide solution, make up to volume, and determine the dextrose present in a 50 cc. portion of the filtrate as directed in the Munson and Walker method, detailed in the chapter on sugars, Chapter IX. If much dextrose is obtained, the Lane-Eynon volumetric method may be used. The weight of dextrose multiplied by 0.9 equals the weight of starch.

Horse Meat

The definition of meat as given by the U. S. Department of Agriculture excludes the use of horse meat unless labeled as such. In certain communities the use of horse meat without declaration is considered not only misbranding but also adulteration. Horse meat unmixed with other meats can be detected and identified by microscopic examination of the flesh structure and by a determination of the constants of the intramuscular fat. The iodine value of the intramuscular fat of horse flesh is much higher than that of beef and considerably higher than that of lard. Thus the respective values are:

lard	47—66.5
beef tallow	35.4—42.3
mutton tallow	48—61
horse fat	75—86

In living animals, the glycogen content of the muscle is intimately connected with the movements of the muscle. In horse muscle, the amount of glycogen is generally greater than that found in other animals and consequently the presence of comparatively large amounts of glycogen in meat or meat products is evidence of horse meat. However, it must be remembered that glycogen is transitory, being broken up into simpler substances, and consequently may not appear in the meat unless examined immediately after slaughtering the animal. Furthermore, glycogen is stored in the liver of animals and therefore the liver of all animals is likely to have comparatively large amounts of glycogen. If the meat is comminuted and composed of different species the difficulties of detecting horse flesh are greatly increased.

Glycogen—*The Brautigam and Edelmann test* [16] is based, as is the following test on the production of a red coloration with iodine solution. 50 g. of the finely divided flesh is boiled for an hour with four times the volume of water. After cooling, add dilute nitric acid to the resulting broth to precipitate the proteins and decolorize the liquid. Filter, and test a portion of the filtrate with a freshly, prepared, saturated aqueous solution of iodine, which is added so as to form a supernatant layer on the surface of the liquid. The presence of glycogen is shown by the production of a wine-red ring at the liquid junction. If the color does not appear, or is uncertain, it may be confirmed by heating the portion to be tested with sufficient potassium hydroxide solution so as to have 3 per cent potassium hydroxide in the resulting mixture, on a water bath until the muscular fiber is decomposed. The broth is concentrated to half its volume, the proteins precipitated with nitric acid and the iodine solution is added as directed above.

Qualitative test: Boil 50 g. of the macerated sample with 50 cc. of water for 15–30 minutes. Filter the broth through moistened filter paper or fine linen. To a portion of the filtrate in a test tube add a few drops of a mixture of 2 parts of iodine, 4 parts of potassium iodide and 100 parts of water. If a considerable quantity of glycogen is present, it produces a dark brown color; this color is destroyed by heating, but it reappears on cooling. If starch is present, it may be precipitated by treating the water extract with two volumes of glacial acetic acid and after filtering applying the test for glycogen to the filtrate.

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[16] Thurston, "Pharmaceutical and Food Analysis," Van Nostrand (1922).

Quantitative estimation:[17] Weigh by difference 25 g. of the finely ground and thoroughly mixed sample and transfer to a 400 cc. beaker. Mix with 50 cc. of potassium hydroxide solution (1.5:1). Cover the beaker with a watch-glass and digest on a steam bath for 2 hours, stirring occasionally. At the end of the 2 hours, dilute to approximately 200 cc. with cold water. Add to the solution an equal volume of 95 per cent alcohol, cover with a watch-glass and set aside overnight. Decant the supernatant liquid through a large folded filter, allowing the glycogen to remain in the beaker, and wash by decantation four times with a mixture of two volumes of 95 per cent alcohol and 1 of water or until the glycogen is white, or nearly so. Transfer the washed precipitate from the beaker to the filter and wash 2 or 3 times more with the solvent mixture. The albuminous substance present retards the filtration if it is permitted to dry on the paper, therefore the funnel should be covered with a watch-glass to prevent excessive evaporation.

After the washing is completed, close the bottom of the funnel by a piece of rubber tubing and a pinch cock. Fill the funnel with warm water, cover with the watch-glass, and let stand overnight. Open the pinch cock and allow all the solution to pass through the filter into a beaker. Close the funnel with the pinch cock and fill with warm water again. Allow the water to remain in the funnel for 1 hour and then filter as before. At first the glycogen solution appears quite turbid. Continue washing with warm water until the filtrate becomes perfectly clear. To the solution of glycogen in water, add double its volume of 95 per cent alcohol and let stand overnight to complete reprecipitation of the glycogen. Filter and wash as before with the solvent mixture.

The last filtration may be made through a tared Gooch crucible and the weight of the glycogen determined by drying to constant weight. This procedure gives results which are approximately correct. More satisfactory results are obtained by hydrolyzing the glycogen with hydrochloric acid (1:3) and determining the resultant dextrose. Dissolve the glycogen on the filter in warm water as directed above, collecting the filtrate and washings in a 300 cc. volumetric flask and keeping the volume within 225 cc. Add 12.5 cc. hydrochloric acid to the combined filtrate and washings, mix, place in a boiling water bath for 3 hours, cool and neutralize with sodium hydroxide solution. Cool again, make up to volume with water and estimate dextrose in an aliquot as directed in the chapter on sugar foods. The corresponding weight of dextrose $\times$ 0.9 = its equivalent

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[17] Trowbridge and Francis, *Ind. Eng. Chem.*, 2, 21, 215 (1910).

of glycogen. Correct this result for dilution to obtain the percentage glycogen in the sample.

This method is based on the separation of glycogen from interfering substances by dissolving those materials in potassium hydroxide solution and alcohol, in which mixture, glycogen is insoluble. The glycogen is subsequently estimated either by direct weighing or by hydrolysis to dextrose which is then determined in the usual manner.

Detection of Added Gelatin in Smoked Meat Products

Pumped meats are sometimes injected with a solution of gelatin. The gelatin solution solidifies during storage and aids both in giving added resistance to knife-cutting of the meat and in the retention of water. In smoked tongues, this addition may be detected organoleptically by cutting the tongue perpendicularly to the veins and then examining the veins. The presence of gelatin will be evidenced by the expulsion of the jelly-like mass from the veins, themselves, by pressure. An aqueous solution of the jelly, after precipitation of proteins, may then be subjected to confirmatory tests for gelatin as described under cream, Chapter VI. Viscolized fats will also be present in the veins if injected and may be analyzed as directed in Chapter VIII.

A modification of the Jacobs and Jaffe method may be used to detect the gelatin in the meat, itself. Weigh 25 g. of the ground sample into a small beaker and add 50 cc. of water. Stir at 5 minute intervals for 20 minutes and then filter through a wire cone or skimmer or through a rapid filter and disregard any cloudiness. Evaporate the filtrate to 10 cc. Cool. Add 3 cc. of lead nitrate solution, 250 g. lead nitrate dissolved in water and made up to 500 cc., and stir. Add 3 cc. of 5 per cent sodium hydroxide solution and stir vigorously. Allow to stand for 5 minutes and filter. To 3 cc. of the filtrate add 2 drops of nitric acid and then a few drops of freshly or recently prepared 5 per cent tannic acid solution. The presence of gelatin is indicated by a white or brownish voluminous precipitate. In the absence of gelatin the solution remains clear. To another portion of the filtrate, add an equal volume of saturated picric acid solution. A marked turbidity indicates the presence of gelatin.

Soy Bean Flour

In a preceding section it was mentioned that soy bean flour is used as a binder and sometimes as a substitute for meat in the manufacture

of sausages. It is of course desirable to be able to tell when such substitution is made. Kerr[18] recommends the following methods. 1) Mix approximately 0.5 g. of the sample with 5 cc. of a solution of urea, 20 g. per liter, in a small test tube or flask containing a strip of red litmus paper partially immersed in the liquid. Stopper the tube or flask and hold at 40° C. for 3 hours. Appearance of a blue color in the litmus paper indicates soy bean flour. This test depends on the presence of the enzyme urease which normally occurs in soy bean flour. This test is not reliable with products which have been heated to a temperature sufficiently high to destroy the urease.

2) Digest 10 g. of the sample in a 150 cc. beaker, covered with a watch-glass, with 50 cc. of alcoholic potash solution, 80 g. stick potassium hydroxide in one liter of 95 per cent alcohol, with occasional stirring to facilitate digestion. Transfer to a 100 cc. graduated oil tube (A. S. T. M. form) or other similar pointed tube, washing the sediment from the beaker with a stream of 95 per cent alcohol from a wash bottle. Bring the volume to 100 cc. with 95 per cent alcohol. Mix, and allow the tube to stand for an hour or more, giving it a gentle rotation from time to time to assist sedimentation. Syphon off the supernatant alcoholic potash solution and examine the sediment microscopically with a magnification of 120–150 diameters. Use a cover slip to cover the preparation. If the sediment is transferred by means of 15–20 cc. of water to a small centrifuge tube and centrifuged, a much better preparation is obtained. Instead of centrifuging, allow the oil tube to stand after mixing with the water until sedimentation is complete or filter, if desired. Look for the large hour-glass or I-shaped cells (sometimes called bearer cells) characteristic of soy beans. Examine with polarized light. The cells stand out quite brilliantly in polarized light. A volume of sediment materially exceeding 0.5 cc., due to spices, in a product containing no starch, except spice starch, warrants suspicion that soy bean flour has been used. Identification of the characteristic soy bean cells in the sediment is proof that soy bean flour has been used.

Neither cooking the frankfurter in the casing as is usually practiced, nor smoking affects the qualitative test for soy, based on the liberation of ammonia from urea by the urease, naturally present. If, however, the soy flour is first made into a stiff paste and heated above 100° C. or under pressure, all of the urease is destroyed.[19] If then, sausage has

[18] Kerr, *J. Assoc. Official Agr. Chem.* **19,** 410 (1936).

[19] LaWall and Harrisson, *J. Assoc. Official Agr. Chem.* **18,** 644 (1935).

been heated or processed in such a way as to destroy not only the characteristic enzyme urease but also to destroy the structure of the characteristic cells of soy bean, it becomes necessary to examine the meat product for other characteristic constituents of soy bean flour. As noted in Chapter X, the soy bean flour is composed roughly of 40 to 45 per cent protein, 20 per cent oil, 6 to 7 per cent ash, 7 to 10 per cent moisture. The remaining material is carbohydrate in nature.

Examination of the ether extract of the meat product will readily show the presence of an oil not normal to meat if a soy bean flour, not defatted, has been used in large amounts. The lecithin content of soy bean flour is given by various investigators as 1.65—3.0 per cent of the flour. The carbohydrate of soy bean consists largely of indigestible material. A high content of crude fiber in a meat product is evidence of the substitution of soy bean for other flours which have only a small percentage of crude fiber. A high carbohydrate content, a high crude fiber content, a high lecithin content, a low non-protein nitrogen content and changed constants of the fat are indicative of soy bean flour.

FISH

The proximate analysis of fish may be made by methods previously described. Thus moisture or conversely total solids, ash and nitrogen may be ascertained as described in an appropriate method in Chapter I. Fat or oil may be estimated by the rapid acid hydrolysis method described for meat or by one of the methods detailed below. Metals, preservatives or added color may be detected as detailed in the chapters concerned with those matters.

Oil

Fish oils are difficult to extract quantitatively from fish flesh because they are easily oxidized and polymerized. The fish flesh may be dried by the vacuum desiccator method and then the fat may be estimated. Stansby and Lemon [20] recommend the following methods: one, rapid and depending on the extraction of oil by shaking with an oil solvent and a dehydrating agent but only approximately quantitative, the other a quantitative continuous acetone extraction method.

Rapid method: Twenty g. of the finely ground flesh, free of skin and bones, is weighed into a shaking bottle equipped with a cork stopper. Add

[20] Stansby and Lemon, *Ind. Eng. Chem., Anal. Ed.* 9, 341 (1937).

25 g. of anhydrous sodium sulfate and exactly 100 cc. of ethyl ether. Shake the bottle and its contents for 60 minutes. Allow most of the fine solid particles to settle out and transfer a 20 cc. aliquot of the solution by means of a pipette, through a filter into a small weighed beaker. The filter is washed with three 3 to 5 cc. portions of ether. The ether is evaporated off on a water bath and the oil is dried for 1 hour at 100° C. and then weighed.

Acetone Continuous Extraction Method: A 15 to 20 g. sample of the finely ground fish flesh is placed in a continuous extractor and heated for 16 hours, replacing the solvent with fresh acetone after 2 hours. The solutions are then heated on a steam bath until all of the acetone and most of the water is removed. They are then placed in a vacuum desiccator over freshly boiled sulfuric acid and the desiccator is evacuated. When practically all of the water has been removed, as indicated by the cessation of foaming and bubbling or after about 3 hours, the oils are removed, and 35 cc. of ethyl ether is added. After thorough shaking, the solution is poured through a filter, preferably one of the sintered-glass type. The residue is washed with several small portions of ether until the wash liquid is colorless, pouring the solution in each case through the filter. The oil solution is washed into a weighed beaker and the ether removed by means of a carefully regulated blast of air. When the odor of ether is no longer evident, the beaker is placed in an oven at 100° to 105° C. for 45 minutes. The beaker and its contents is then cooled and weighed.

By these methods, Stansby and Lemon established that the average value of oil in mackerel varied from 12 to 15 per cent rather than the value of 7.1 per cent given in Table 63.

Detection of Decomposition in Fish

Rancidity in fish may be detected organoleptically for it is generally evident by an after taste. Untreated fish when decomposed will have the usual accompanying bad odor and appearance but smoked fish will not show rancidity as readily. Wheeler [21] determines the peroxide number of oils and this number may be used as an index of rancidity.[22]

Pipette a 20 cc. aliquot of the ether solution of the oil obtained as described in the preceding section, "Rapid Method," into a 500 cc. flask.

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21 Wheeler, *Oil and Soap* **9**, 89 (1932).

22 Stansby, *J. Assoc. Official Agr. Chem.* **18**, 618 (1935).

TABLE 63. PROXIMATE COMPOSITION OF FISH [23]

Type	Moisture	Protein	Fat	Ash
Bass	77.7	18.6	2.8	1.2
Cod	82.6	16.5	0.4	1.2
Flounder	84.2	14.2	0.6	1.3
Haddock	81.7	17.2	0.3	1.2
Halibut	75.4	18.6	5.2	1.0
Herring	72.5	19.5	7.1	1.5
Mackerel	73.4	18.7	7.1	1.2
Perch, white	75.7	19.3	4.0	1.2
Perch, yellow	79.3	18.7	0.8	1.2
Pickerel, pike	79.8	18.7	0.5	1.1
Salmon	64.6	22.0	12.8	1.4
Shad	70.6	18.8	9.5	1.3
Smelt	79.2	17.6	1.8	1.7
Sturgeon	78.7	18.1	1.9	1.4
Trout	77.8	19.2	2.1	1.2
Whitefish	69.8	22.9	6.5	1.6

Add 50 cc. of a freshly prepared mixture of 60 per cent glacial acetic acid and 40 per cent chloroform, followed immediately by 1 cc. of a saturated solution of potassium iodide from a pipette. Shake the flask with a rotary motion for exactly 1 minute. Quickly add 100 cc. of a 0.05 per cent starch solution. Immediately titrate the solution with 0.01 *N* sodium thiosulfate solution to the disappearance of the last purple tinge, shaking vigorously at the end point. Express the peroxide number, *M*, as the millimoles of peroxide per 1000 g. of oil. The weight of the oil in the 20 cc. aliquot is known from the previous determination. Then,

$$M = 0.5 \frac{\text{(cc. thiosulfate) (normality)}}{\text{weight of oil}} \cdot 1000$$

For example, suppose one 20 cc. portion of ether solution contained 0.407 g. of oil and a second 20 cc. aliquot of the ether solution required 3.90 cc. of 0.009 *N* sodium thiosulfate solution, then the peroxide number,

$$M = \frac{(0.5)(3.90)(0.009)}{0.407} = 0.0431$$

This is the value for 1 g. of oil, hence multiplying by 1000 gives the desired value, namely, 43.1.

[23] Atwater and Bryant, U. S. Dept. Agr., Bull. No. 28 (1906).

Estimation of free fatty acids serves as a qualitative test for, as decomposition of oils increase, the free fatty acid content also increases.

In mackerel peroxide values and organoleptic examination give the following groups:

not rancid	0.0 to 0.6
slightly rancid	0. to 21.4
rancid	18.4 to 36.5
extremely rancid	33 to 201

Chloride[24]

Chloride expressed as sodium chloride may be estimated by simple titration with thiocyanate solution with but slight modification. Place 10 g. of the sample into a flask or beaker, add a known volume of silver nitrate solution which is more than sufficient to precipitate all the chloride as silver chloride and then add 20 cc. of nitric acid. Boil gently on a hot plate, until all solid matter is dissolved except silver chloride. Cool; add 50 cc. of water; 5 cc. of a saturated solution of hydrazine sulfate, to remove any nitrous acid formed; 5 cc. of a saturated solution of ferric ammonium alum as indicator; 1 cc. of nitrobenzene for each 0.05 g. of chloride[25]; and titrate the excess of silver with 0.1 *N* potassium thiocyanate solution until a permanent light brown color appears. Subtract the cc. of 0.1 *N* thiocyanate used from the cc. of 0.1 *N* silver nitrate added and calculate the quantity of chlorine as sodium chloride. With a 10 g. sample, each cc. of 0.1 *N* silver nitrate solution is equivalent to 0.058 per cent sodium chloride.

If it is desired to make the determination on an ash, add 10 per cent calcium acetate solution as the fixative and ash at 550° C. Dissolve the ash in 25 cc. of nitric acid (1:3) and proceed as directed above.

OYSTERS

It is generally sufficient for the chemical determination of "floating" or excessive washing of oysters to estimate the solids, ash and salt content of oysters. "Floating" is the practice of soaking oysters, fish and the like, in fresh water for the purpose of making them appear plump

24 Grigsby, *J. Assoc. Official Agr. Chem.* 20, 410 (1937)

25 Caldwell and Moyer, *Ind. Eng. Chem., Anal. Ed.* 7, 38 (1935).

and increasing their weight. Since the oysters have a higher salt content than the surrounding fresh water, the water passes into the oysters by the process of osmosis and thus bloats them.

Where oysters are grown on muddy or soft bottoms, the shells often become coated with mud. The most efficient method of washing such oysters, and for that matter, all oysters, is by using a blower with a solution of salt. If the oysters are subjected to a reasonable washing with fresh water, a gain in volume from 3 to 10 per cent may take place.[26]

The volume of free liquor in oysters may be ascertained by draining the meats on an oyster skimmer for 1 minute and measuring the shrinkage in volume of the meats after they are returned to the original measuring vessel. The skimmer is a metal tray with holes 1/4 inch in diameter, spaced 1 1/4 inches in a square pattern and of sufficient area to take the entire volume of oysters drained at one time in a single layer.[27]

Atwater[28] and Bryant give the proximate composition of oysters as: total solids—13.1%; protein—6.2%; fat—1.2%; carbohydrate—3.7%; ash—2.0%. More recent analyses are tabulated in Table 64.

Table 64. New England Oysters[29]

	Unwashed			Washed		
	Max.	Min.	Ave.	Max.	Min.	Ave.
Meat	95.7	75.6	85.1	98.5	86.1	93.1
Liquor	24.4	4.3	14.9	13.9	1.5	6.9
Meats						
Solids	24.05	16.94	20.57	21.44	15.29	13.20
Ash	3.03	1.84	2.29	1.99	1.20	1.50
Salt	1.41	0.67	0.92	0.63	0.24	0.35
Liquor						
Solids	6.67	4.60	5.32	7.00	3.62	5.01
Ash	2.76	2.40	2.57	1.72	1.08	1.34
Salt	2.66	1.81	2.13	1.56	0.49	0.97
Entire Sample						
Solids	21.58	15.55	18.29	21.0	14.41	17.28
Ash	2.70	1.97	2.25	1.77	1.25	1.44
Salt	1.53	0.80	1.10	0.68	0.27	0.40

[26] Hunter and Harrison, U. S. Dept. Agr., Tech. Bull. No. 64 (1928).
[27] Grigsby, *J. Assoc. Official Agr. Chem.* 20, 410 (1937).
[28] Atwater and Bryant, U. S. Dept. Agr., Bull. No. 28 (1906).
[29] Hunter and Harrison, U. S. Dept. Agr., Tech. Bull. No. 64 (1928).

EGGS AND EGG PRODUCTS

Eggs are almost as complete a food as milk although not as well balanced.

Definitions.

The Food and Drug Administration gives the following definitions for eggs and egg products.

1) Liquid eggs, mixed eggs is the product obtained by separating the edible portion of eggs from the shells. It is an intimate mixture of the whites and yolks in their natural proportions.

2) Frozen eggs is the solidified product obtained by quickly and completely freezing liquid eggs.

3) Dried eggs is the product obtained by evaporating the water from liquid eggs. It contains not more than 7 per cent of moisture.

4) Egg yolk is the product obtained by removing the whites from the yolks in the commercial process of egg-breaking. It contains not more than 12 per cent by weight of adhering white.

5) Frozen egg yolk is the solidified product obtained by quickly and completely freezing egg yolk.

6) Dried egg yolk is the product obtained by evaporating the water from egg yolk. It contains not more than 5 per cent of moisture.

Whole Eggs

Whole eggs are seldom analyzed chemically by the food analyst except for investigational purposes. However, organoleptic examination by "candling," that is examination of the egg by rotation before a light, is often necessary. Whole eggs are generally classified as follows:

1) Firsts and extras are freshly laid, sound, whole, clean shell, medium and good-sized eggs. The best eggs of the year are laid during the spring months and these eggs are usually selected for storage for the winter trade.

2) Seconds include all eggs not considered firsts except "spots" and "rots." Generally this group includes all the undersized, dirty, checked, cracked shell, or weak eggs. These terms signify:

a) Undersized are eggs which may be classed as first except for size.

b) Checks and Cracks are eggs, the shells of which have become broken by careless handling, but which have shell membranes intact.

c) Leakers are eggs whose shells and shell membranes are sufficiently broken to permit a portion of their contents to escape.

d) Dirties are eggs, the shells of which have become soiled from unclean nests and through other means.

e) Weak eggs are those in which the albumin has become weak or watery due to high or varying temperature changes.

These seconds are considered suitable for food purposes when handled under proper conditions, except "leakers" which are usually badly contaminated.

3) Spots and rots consist of all the discards and are considered useful only for manufacturing purposes, such as for tanning leather. They are eggs which show a spot before the candle. These spots may be due to developing embryos, blood rings, or molds, or by the attachment of the yolk to the shell, which may be caused by holding in one position. White rot or sour rot designate eggs whose white and yolk are just beginning to mix. Stale eggs are those whose shell shows an enlarged air space and whose yolk has gained in opacity and fallen to the pointed end of the shell. Spot rot designate eggs in which the yolk becomes adherent to one or both of the shell membranes. Moldy eggs are those which show dense black areas of varying size inside the shell. Light spot eggs are those which have been incubated sufficiently to show a small darkened area.

Analysis of Eggs

The rapid growth of the egg-breaking industry has led to increased need for accurate and reliable laboratory methods of analysis and control, especially because eggs are a product that is highly perishable and cannot be pasteurized as are milk, cheese and similar dairy products. Preservation is accomplished generally by means of rapid or sharp freezing and any changes in composition of the product packed must be made within a few hours, or before the batch in question is frozen solid. The eggs must be packed in different compositions for different industries. Thus egg whites are used by the baking and candy industries; plain yolk and salted yolk by the mayonnaise and noodle trades; whole eggs, sugar yolk, and glycerol yolk by the baking, ice cream, and confectionery trades. Special types of egg products are packed to specification.

Guthmann and Terre [30] recommend that in order to overcome discrepancies in sampling at least 3 samples be taken from each batch

[30] Guthmann and Terre, *Ind. Eng. Chem., Anal. Ed.* **8**, 377 (1936).

churned, one at the top of the churn, one at the middle and one at the bottom. The three samples are then mixed in a quart container such as a mason jar and constitute a composite sample. Frozen eggs in 30 lb. cans may be sampled by the use of a drill in a manner similar to the use of a trier as describer in Chapter I.

In general, analyses may be made in a manner similar to that of other food products. Total solids are most accurately determined by the prior evaporation to dryness of a weighed portion in a tared dish with final drying in a vacuum oven. Bailey [31] gives a refractometric method for liquid eggs, which is, of course, useful for rapidity although not yet completely satisfactory. Fat may be determined on an appropriate weight of sample by the acid hydrolysis modification of the Roese-Gottlieb method detailed under the section "Cheese". Nitrogen may be determined by the Kjeldahl-Gunning-Arnold method. Ash, phosphates, after making alkaline with 10 per cent sodium carbonate solution, are estimated as usual.

Lipoids and Lipoid Phosphoric Acid

The A. O. A. C. gives the following directions for this determination.

Reagents: a) Mixed solvent. Equal volumes of chloroform and absolute alcohol.

b) Alcoholic sodium hydroxide. Prepare a saturated solution free from carbonates by dissolving 100 g. of sodium hydroxide in 100 cc. of water. Allow the mixture to stand until clear, or filter through a hard filter paper which has been soaked in alcohol. Five cc. of the sodium hydroxide solution contains approximately 4 g. of NaOH. Dissolve 50 cc. of this solution in 900 cc. of 95 per cent alcohol and dilute with 95 per cent alcohol to 1 liter.

Preparation of solution: a) Liquid eggs. Weigh accurately by difference (a Mojonnier pipette and carriage are useful) approximately 4 g. of the well mixed sample into a 100 cc. volumetric flask, add very slowly, dropwise, from a pipette, 25 cc. of the mixed solvent, shaking constantly until the proteins become coagulated and then thoroughly broken up. Add 60–65 cc. more of the solvent and allow to stand 1 hour, shaking at 5 minute intervals. Fill to the mark with the solvent, shake, and allow the mixture to stand until clear.

b) Dried eggs. Transfer 2 g. of the well-mixed sample into a 100 cc.

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[31] Bailey, *Ind. Eng. Chem., Anal. Ed.* 7, 385 (1935).

volumetric flask, add 85–90 cc. of the mixed solvent and allow to stand 1 hour, mixing at 5 minute intervals. Proceed as directed above.

Determination of lipoids: Transfer a 50 cc. aliquot to a 150 cc. beaker and evaporate the extract to dryness on a steam bath. An electric fan or a gentle blast of dry air may be used to hasten evaporation. Place the beaker into an oven at 100° C. for 5–10 minutes to remove any remaining moisture. Dissolve the dry extract in 5–10 cc. of chloroform and filter the solution into a weighed 100 cc. Pyrex beaker through a pledget of cotton packed into the stem of a funnel, transferring all soluble extract from the bottom and sides of the beaker by means of chloroform from a wash bottle. Finally wash the funnel and stem tip. The filtrate should be clear. Evaporate the chloroform on a steam bath, and dry the beaker and contents in an oven at 100° C. to minimum weight, approximately 90 minutes. Allow the beaker to stand in air until no further change in weight takes place, approximately 30 minutes. Weigh, and report the percentage of lipoids.

Determination of lipoid phosphorus pentoxide: Dissolve the dried lipoids in 2–3 cc. of chloroform, add 10–20 cc. of the alcoholic sodium hydroxide solution, evaporate to dryness on a steam bath, using care to avoid spattering, and place the beaker into an oven at 100° C. for 30 minutes to remove any remaining moisture. Transfer the beaker while hot to an electric muffle heated to 500° C., faint redness, and allow it to remain at that temperature for 1 hour. Cool, add a few drops of water, break up the charge with a glass rod, having a flattened end, cover the beaker with a watch-glass, add slowly 5 cc. nitric acid (1:3), mix, remove the watch-glass, and filter, collecting the filtrate in a 300 cc. or 500 cc. flask. Thoroughly wash the charred material and filter paper with water, from a wash bottle. Determine phosphorus pentoxide as directed in Chapter XVII. Report as percentage lipoid phosphorus pentoxide.

Distinction Between Egg Yolk and Vegetable Lecithin

With the increased use of soy bean flour and of vegetable lecithin, itself, which is used as an emulsifying agent, the estimation of lecithin-phosphorus pentoxide as a means of determining egg content is dubious unless assurance that vegetable lecithin is not present is made. Differences in the nitrogen-phosphorus ratio are used by some investigators.[32] Kluge [33] uses the lecithin-phosphorus to sterols ratio which is greater in

[32] Nottbohm and Mayer, *Chem. Ztg.* **56**, 881 (1932).

[33] Kluge, *Z. Untersuch. Lebensm.* **69**, 9 (1935).

egg yolk than in vegetable lecithin. Because this ratio is very much greater in egg yolk than in vegetable lecithin preparations, it may be utilized in conjunction with other tests for the detection of vegetable lecithin in food pastes. If the egg content of the material, as calculated from the lecithin-phosphorus content is greater than that calculated from the sterols present, the presence of a vegetable lecithin preparation is indicated.

A qualitative test is Weyl's [33] lutein test. The dry residue from an acetone extraction is dissolved in ether and treated with solid sodium nitrite and dilute sulfuric acid. With the oil from egg food pastes, the deep yellow etheral solution undergoes almost complete decolorization, whereas with that from vegetable lecithin the yellow color persists virtually unchanged.

Another test is that the iodine value of egg lecithin is lower than that of vegetable lecithin. The former has a value about 70 whereas the latter has a value from 108 to 117.

Glycerol

Glycerol yolks are packed chiefly for the baking trade. They contain from 5–7 per cent of glycerol and have the property of keeping the cakes fresh and moist for a longer period of time. The following method is not applicable in the presence of sugar in the yolks. The reagents used are those detailed in the section "Glycerol," Chapter XII.

Weigh out from 8 to 10 g. of the sample into a 50 cc. beaker, add 1 cc. of 5 per cent acetic acid, and heat over a low flame on a wire gauze with constant stirring until the egg is thoroughly coagulated. The material must not be allowed to char. The lumps of egg should be dry in character and not of a sticky or pasty consistency.

Transfer the coagulated material to a 250 cc. volumetric flask, washing the beaker with 25 to 50 cc. of water. Add about 100 cc. of washed alumina cream and make up the mixture to the mark with water; shake thoroughly, allow to stand for 5 minutes, shake again, and filter through a coarse filter paper. The filtrate should be perfectly clear. Transfer 25 cc. of the filtrate to a 250 cc. volumetric flask and add 25 cc.of sulfuric acid and 2 cc. of silver nitrate, 10 per cent solution. Add 10 cc. of a strong potassium dichromate solution, slowly from a pipette at 20° C., place the volumetric flask on a steam bath, and heat for exactly 30 minutes. Cool and dilute to the mark with water. Place this solution in a burette and titrate into a beaker containing 100 cc. of water, 15 cc.

of sulfuric-phosphoric acid retarding mixture and 10 cc. of ferrous ammonium sulfate solution measured accurately with a pipette. Add 3 drops of a solution of 1 g. of diphenylamine in 100 cc. of sulfuric acid, as indicator.

As the titration proceeds the color changes from green to blue-gray to a pure violet at the end point.

Before each determination the ferrous ammonium sulfate solution is standardized against dilute dichromate solution. Measure 25 cc. of strong dichromate solution at 20° C. into a 500 cc. volumetric flask and dilute to the mark with water at room temperature. Twenty cc. of this solution equals 1 cc. of strong dichromate. Place the dilute dichromate in a burette, and titrate into a beaker containing 100 cc. of water, 15 cc. of sulfuric-phosphoric acid mixture, and 10 cc. of the ferrous ammonium sulfate. Three drops of diphenylamine solution are used as an indicator just as in the determination.

The percentage of glycerol is calculated according to the following formula:

When 10 cc. of strong dichromate solution and 10 cc. of ferrous ammonium sulfate solution are used

$$\%\text{ of glycerol} = \frac{10\left[10.0 - \left(\dfrac{12.5 \times \text{cc. of dilute } K_2Cr_2O_7}{\text{cc. of oxidized glycerol solution}}\right)\right]}{\text{sample weight}}$$

There is some oxidizable material present in ordinary egg yolk, hence the determination of glycerol in a prepared glycerol yolk is on an average 0.5 per cent high. Controls with pure egg yolks should be run at the same time or else the above mentioned figure should be deducted.

Glycerol in Presence of Sugar[34]

Proceed as detailed in the glycerol determination, taking a sample so that the combined weight of glycerol and sugar is not more than 3.0 g. If starch is present, it must be removed by the usual alcohol procedure or by some other method. If alcohol is used, the alcohol should be removed by evaporation. Boil for 20 minutes with the sulfuric acid in order to insure complete inversion. Determine the amount of potassium

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[34] Hoyt and Pemberton, *Cotton Oil Press* **14**, 54 (1922); **14**, 340 (1922).

dichromate needed to oxidize both sugar and glycerol. Determine invert sugar as detailed in the next section. Calculate percentage of glycerol after deducting the amount of potassium dichromate required by sugar.

1 cc. $K_2Cr_2O_7$ is equivalent to 0.0100 g. of glycerol

1 cc. $K_2Cr_2O_7$ is equivalent to 0.01142 g. of invert sugar.

The sugar may be removed with milk of lime as described in the section, "Glycerol," Chapter XIV and the glycerol may then be estimated as previously described.

Sugar

Sugar before and after inversion may be estimated by the Lane-Eynon method. Wash about 25 g. of the sample into a 200 cc. volumetric flask with 75 cc. of water. Make slightly acid by adding 2 cc. of 5 per cent acetic acid for white or whole egg and 1 cc. for yolk. Mix, and immerse the flask in boiling water until the egg material is thoroughly coagulated. This requires from 15 minutes to 0.5 hour. Cool to room temperature and make up to the mark with washed alumina cream. Shake the sample vigorously for 1 minute, allow it to stand for 5 minutes, and then shake for 1 minute. Filter through a dry folded filter. Dilute 25 cc. of the filtrate to 100 cc. and proceed to determine reducing sugars before inversion by the Lane-Eynon method as detailed in Chapter IX. Invert 25 cc. of the filtrate by the hydrochloric acid inversion method, make to volume after neutralization and proceed with the Lane-Eynon method to determine total reducing sugars.

Salt

Salt yolks are extensively packed for the mayonnaise trade, since the presence of about 10 per cent salt not only acts as a preservative but permits the egg to be thawed as a syrupy liquid, whereas a plain yolk when thawed has a gummy, semi-solid consistency. Since salt is very much cheaper than egg yolks, both the packer and the consumer are vigilant in seeing that the amount of salt added stays very close to the predetermined figure.

Weigh 5 g. of the sample into a 500 cc. volumetric flask containing 200 cc. of water. Make up to the mark and shake thoroughly. Pipette 50 cc. of this solution into an Erlenmeyer flask, and add 10 cc. of 0.1 *N* silver nitrate solution with shaking. Add 10 cc. strong nitric acid and 5 cc. of a saturated solution of ferric ammonium sulfate, and titrate with 0.1 *N* ammonium thiocyanate solution to a permanent light brown color.

One cc. of 0.1 *N* silver nitrate equals 0.0058 g. of sodium chloride. Correction factor: Subtract 0.3 per cent of sodium chloride from the result.

REFERENCES

Allen's Commercial Organic Analysis, 5th ed., Vol. IX, Blakiston (1932).
Leach-Winton, "Food Inspection and Analysis," Wiley (1920).
Thurston, "Pharmaceutical and Food Analysis," Van Nostrand (1922).
Methods of the A. O. A. C. (1935).
Hawk and Bergeim, "Physiological Chemistry," Blakiston (1926).
Moulton, "Meat through the Microscope," Chicago (1929).

CHAPTER XVI

VITAMINS

Classification

Vitamins are substances that are necessary in the diet for proper growth and for the prevention of disease. Recently eight substances or factors have been recognized and are subject to either chemical, physico-chemical or biological assay. These are A, B_1, C, D, E, F, G, and B_2. Some of the substances in these eight factors have been isolated in crystalline form, as for example, ascorbic acid or vitamin C, aneurin or vitamin B (B_1). Others have been concentrated so that a small portion of the concentrate has an enormous potency in terms of biological effect.

The more or less well characterized vitamins, their metabolic effect, and some of the foods in which they occur are detailed below.

Vitamin A, the anti-ophthalmic (anti-xerotic) vitamin is oil soluble. It prevents ophthalmia, a disease causing inflammation of the eye and recent investigational work indicates that it aids in the prevention of infections due to a break-down of mucous membranes. It also prevents night-blindness.[1] The breakdown of the mucous membrane caused by lack of the vitamin provides a base for local infections, which sufficient vitamin A prevents. It is also a growth factor. It has no effect in preventing or curing, except with respect to the above, septicemia, common cold, pneumonia, scarlet fever or tuberculosis. It is found in butter and milk and milk products generally, eggs, cod liver and other fish liver oils and in spinach.

Vitamin B (B_1) the antineuritic vitamin is water soluble. It prevents beri-beri, a disease causing paralysis of the nervous system and other nervous diseases. It aids in the prevention of stunted growth and loss of weight. It is found in green vegetables generally, whole grains, tomatoes and in yeast. Apparently vitamin B_1 has its curative effect by acting as a co-enzyme for lactic acid oxidation.

Vitamin C, the anti-scorbutic vitamin, is water soluble. It aids in the prevention and cure of scurvy, a disease causing ulcerated gums and

[1] Harris, "Vitamins," MacMillan (1935).

pains in the joints and muscles. Vitamin C probably works in the body as a reducing agent and thus takes part in the many oxidation-reduction processes of metabolism. It is found in green vegetables, tomatoes, citrus fruits and in other fruits.

Vitamin D, the anti-rachitic vitamin is oil soluble. Vitamin D is not a single substance but is the term used to cover a group of substances, all sterols or sterol derivatives, that are antirachitically active. The vitamins D prevent rickets and the subsequent poor development of the bones and teeth. The vitamin is found naturally, mainly, in cod liver oil and other fish liver oils and in lesser amounts in butter and milk products. It is produced commercially by the irradiation of ergosterol and by the irradiation of many food products directly. Vitamin D aids in the metabolic process by helping the body absorb and utilize properly the phosphorus and calcium obtained from food.

Vitamin E, the anti-sterility vitamin is oil soluble. It prevents sterility in males and the resorption of the foetus in the female. It is found, mainly, in the wheat germ, from which two alcohols were isolated showing vitamin E activity.

Vitamin B_2 is generally used to designate the lactoflavin factor of the vitamin G complex of water soluble factors. It is a growth promoting factor, and is found in milk.

The other vitamins are not well characterized and will not be discussed.

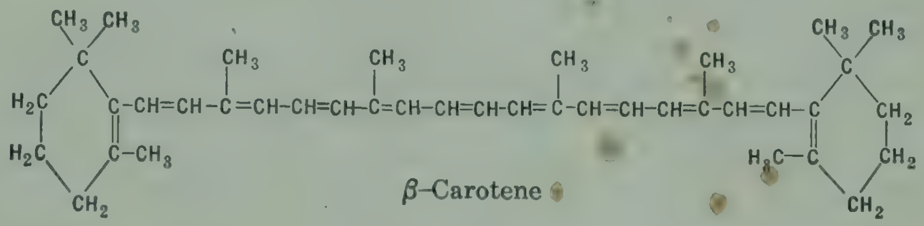
Because of the uncertainty of the chemical nature of some of the vitamins, few completely satisfactory tests have been developed for their detection and estimation. It is necessary that any method based on chemical or physicochemical procedures have a fair degree of correlation with biological assays, otherwise the chemical or physical method is valueless as a measure of the metabolic effect. Chemical methods are of great and growing importance, for as the chemistry of the vitamins becomes clearer and as the various vitamin factors are isolated and synthesized, methods for their estimation follow as a matter of course. Even if the chemical properties are ascertained before isolation, methods can be developed. It must be recognized that some of the biological assays are subject to error and that even approximate chemical methods are valuable adjuncts to biological procedures. Some of the methods that are applicable in a food laboratory and do have a good correlation with biological assays are detailed.

It must not be forgotten that biological methods of assay are sometimes inconsistent in themselves, and therefore, although it is necessary

that some degree of correlation exist between a chemical and biological assay, caution must be observed before assuming that a chemical method is valueless because the degree of correlation with a biological assay is small.

Vitamin A

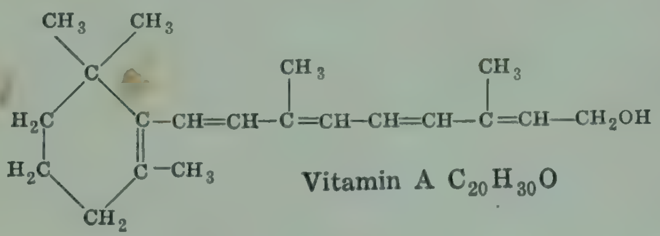
Vitamin A is generally considered to be an oxidation product of half the carotene molecule and is an unsaturated alcohol of high molecular weight. Carotene has been assigned the formula $C_{40}H_{56}$, structurally.[2]



β–Carotene

α Carotene differs from β carotene in that the positions of the double bonds are changed so that the molecule has an asymmetric carbon atom. Carotene has been shown by numerous investigators to give rise to vitamin A in the body and hence it is considered to be provitamin A.

The structural formula for Vitamin A is



Vitamin A $C_{20}H_{30}O$

It was noticed a number of years ago that cod liver oils gave a blue or purple color with dehydrating agents such as sulfuric acid. Rosenheim and Drummond found that arsenic trichloride gave an intense blue color that did not fade rapidly. Carr and Price[3] found that al-

[2] Bacharach and Smith, *Analyst* 59, 70 (1934).

[3] Carr and Price, *Biochem. J.* 20, 497 (1926).

though the color with antimony trichloride was less intense, it was far more stable and hence should serve as a basis for its estimation. On the development of this color, many tests for the quantitative determination of vitamin A were developed.

Antimony Trichloride Method—One gram of oil is accurately weighed into a volumetric flask of 10 cc. capacity. The oil is dissolved in chloroform and is made to volume with this solvent. Transfer 0.2 cc. of this solution with the aid of a pipette to a glass cell of 1 cm. internal thickness, and exactly 2 cc. of a 30 per cent solution of antimony trichloride in chloroform is run in from a burette. During the addition of the reagent the solution is gently shaken. The blue color produced is matched in a Rosenheim-Schuster colorimeter.[4] The final match is made at the point of maximum intensity of the blue color. The blue color is then correlated to a blue value expressed in blue units based on the assumption that the blue value of pure vitamin A is equal to 80,000.

Many observers have found that the biological potency of cod liver oil and other liver oils is far in excess of the chemical blue value and state that the absorption at 328 mμ gives far better agreement with the biological measure of vitamin A. Some investigators determine the blue value of the unsaponifiable fraction of the oil and determine the vitamin A corresponding to this value. However, it is doubtful whether the increase in blue value compensates for the labor involved in obtaining the unsaponifiable residue.

In the Carr-Price method, the chloroform solution of the oil is diluted in such a manner that 0.2 cc. of this solution mixed with 2 cc. of a saturated solution of antimony trichloride in dry alcohol-free chloroform produces a color between 4.0 and 6.0 units of Lovibond blue. The result is then calculated to the proportionate color for 0.04 g. of the original oil. Either daylight or artificial daylight may be used and at the point of maximum intensity red or yellow glasses should be used in addition to blue glasses in order to obtain a proper match.

Although the blue value of an oil does not in any of these methods necessarily yield the true vitamin A content of an oil, nevertheless it is indicative of the approximate vitamin A content. These tests are of great value in establishing the presence or absence of appreciable amounts of Vitamin A.

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[4] Griffiths, Hilditch and Rae, *Analyst* 58, 65 (1933).

Guiacol or Catechol Method—Another method for vitamin A, developed by Rosenthal and Erdélyi,[5] appears to be an improvement over the Carr-Price technique. The oil containing vitamin A is diluted with alcohol-free chloroform in the proportion of 0.1 to 0.5 cc. of oil to 1 cc. of chloroform, according to the vitamin content. To 1 cc. of this solution is added 1 cc. of a freshly prepared 0.5 per cent solution of catechol in chloroform and 3 cc. of a cold saturated solution of antimony trichloride in chloroform. The tube containing the mixture is heated immediately to 60° C. for 1 to 2 minutes. The blue color first produced changes to an intense violet red. Quantitative determinations may be made with the violet red stage by comparison with a 0.01 per cent solution of potassium permanganate. Using guiacol instead of catechol, the color is produced without heating.

Carotene, lycopin, zeaxanthin, capsanthin, physalin, do not give the reaction. Ergosterol turns pink first and then becomes blue. The color produced with guiacol and with catechol is more stable than that produced by the Carr-Price reaction.

Spectrographic Method—It has been shown that the ultra-violet absorption of vitamin A at 328 mμ (3280 Å) is not due to carotene or to similar coloring matters. Thus carotene (British Drug House) absorbs at about 450 mμ, which is in the visible region and its absorption in the ultra-violet is negligible. Calciferol or vitamin D absorbs at about 265 to 270 mμ.[6]

The determinations are made by taking a series of pairs of photographs with the aid of a quartz spectrograph fitted with a short-focus rotating sector photometer mounted directly on the instrument. Each pair of photographs of the spectrum consists of one spectrogram taken through a solution of the substance and another taken through the same thickness of the solvent in a quartz cell. By means of the sector photometer, the time of exposure of the individual halves of each pair of spectrograms is varied so that the relative exposures through the solvent are decreased quantitatively. In this way points can be found on each pair of photographs where the density of the two halves is the same, showing that at these points the intensities of the two beams of light, multiplied by the relative exposures are equal. As the ratio of the exposures decreases, the points of equal density of the spectrograms be-

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[5] Rosenthal and Erdélyi, *Biochem. J.* **28,** 41 (1934); **29,** 2112 (1935).

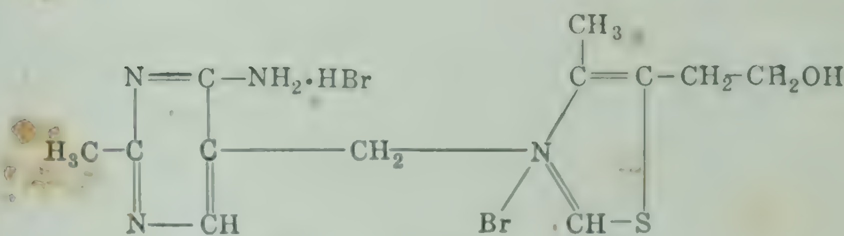
[6] Crews and Cox, *Analyst* **59,** 85 (1934).

come closer together, until at the head of the band they become coincident. This point is used for the calculation of the extinction coefficient, E, which is the logarithm of the ratio of the intensity of the transmitted light to the incident light.

In the case of vitamin A preparations, it is usual to calculate to a 1 per cent concentration and a 1 cm. cell, and to specify the particular wavelength or band at which absorption has taken place. This is usually expressed as $E^{1 cm.}_{1\%}$ 328 mμ. Solvents used must be specifically purified for spectrophotographic work, generally absolute alcohol and cyclohexane are used. $E^{1 cm.}_{1\%}$ 328 mμ for pure vitamin A equals 1600.

Vitamin B (B_1)

Vitamin B usually termed B_1 has been isolated from yeast and other materials rich in the vitamin and has been called by some investigators antineurin, aneurin and thiamin. It was probably first isolated in 1927 by Jansen and Donath.[7] This vitamin has the chemical characteristics of a compound composed of a pyrimidine and a thiazole ring. A hydrobromide of a substance having the chemical and physiological properties of vitamin B_1 has been synthesized by Williams and coworkers.[8] They assign the formula $C_{12}H_{17}N_4SOBr.HBr$ structurally,



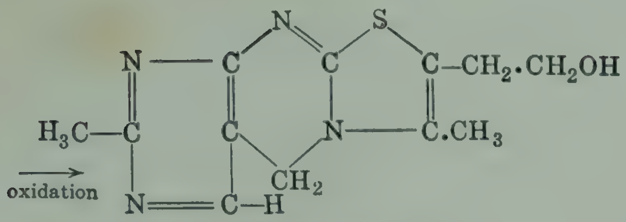
Vitamin B_1 hydrobromide or Aneurin

Thiochrome Reaction—A method for the estimation of vitamin B_1 has been developed on the observation of Barger[9] the vitamin is oxidized to thiochrome, to which has been assigned the formula $C_{12}H_{14}ON_4S$, by

[7] Harris, "Vitamins," Macmillan (1935).

[8] Williams and Cline, *J. Am. Chem. Soc.* **58**, 1504 (1936).

[9] Barger, *Ber.* **68**, 2257 (1935).



Thiochrome [10a]

potassium ferricyanide in alkaline solution. Jansen [10] using this method found good agreement with biological assay.

Ten cc. of a 25 per cent extract of yeast in water at a pH 3 is treated with 20 cc. of methyl alcohol. The precipitate is removed by centrifuging and 1 cc. is mixed with 0.1 to 0.3 cc. of 1 per cent ferricyanide solution, 1 cc. of methyl alcohol and 1 cc. of 30 per cent sodium hydroxide solution. This mixture is then extracted with 13 cc. of iso-butyl alcohol. The two layers are separated by centrifuging and from the upper layer 10 cc. is pipetted into the vessel of the fluorometer. The fluorescence due to the thiochrome is measured by a galvanometer through which the fluctuating current of the photoelectric cell of the fluorometer passes.

Pure aneurin may be assayed in a similar manner. 0.1 cc. containing 1 to 20 gamma is placed in a 25 cc. glass stoppered cylinder (rubber stoppers give rise to fluorescent substances). Add 0.1 per cent solution of potassium ferricyanide in the proportion of 0.01 to 0.1 cc. for 1 gamma, 0.03 to 0.1 cc. for 10 gamma and 0.1 to 0.2 cc. for 20 gamma. Mix and add 3 cc. of 10 per cent sodium hydroxide. Mix again thoroughly and after 1 to two minutes extract with 13 cc. of iso-butyl alcohol. Centrifuge, transfer 10 cc. of the supernatant solvent to the cell of the fluorometer and read.

The International Standard power (adsorbate) may be assayed in a similar manner. Weigh accurately from 10 to 50 mg. of the material and transfer to a 25 cc. glass stoppered cylinder and add 10 cc. of 10 per cent sodium hydroxide solution. Add the requisite amount of 0.1 per cent potassium ferricyanide solution. For 10 mg. take 0.05 to 0.1 cc., for 30 mg. take 0.1 to 0.2 cc. and for 50 mg. take 0.3 cc. of the ferricyanide solution. The mixture is stirred for 5 to 10 minutes after which it is extracted with 13 cc. of iso-butyl alcohol. The fluorescence of the supernatant liquid is read as before in the fluorometer.

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[10] Jansen, *Rec. trav. chim. Pays-Bas* **55**, 1046 (1936).

[10a] Todd, *J. Chem. Soc.*, p. 1601 (1936).

Other methods of estimation of the vitamin are being developed and undoubtedly the determination of the potency and content of materials containing vitamin B_1 will soon be on a fixed basis.

One of these developed by Prebluda and McCollum [11] depends on the formation of a water insoluble compound from the condensation of vitamin B_1 and a diazotized compound. These investigators found that a solution of either p-aminoacetanilide or methyl-p-aminophenylketone (p-aminoacetophenone) treated with nitrous acid yields a diazotized compound which when added to vitamin B_1 under definite conditions yields a characteristic purple red compound which is stable and highly insoluble in water. The reagents prepared from the aforementioned amines were reacted with samples of wheat germ, rice polishings, and a number of commercial preparations. In each case the same characteristic product previously mentioned may be obtained.

Another method correlates the added amount of carbon dioxide, liberated in the fermentation of dextrose, with the quantity of vitamin B_1 present.[12] Determinations are made of the volume of carbon dioxide liberated in 3 hours by the fermentation of dextrose in the presence of vitamin B_1. With known amounts of natural crystalline vitamin B_1, by means of the following method, the tabulated results were obtained.

For each test, 1 g. of yeast, 100 cc. of an aqueous solution containing a synthetic salt mixture as a buffer and 3 g. of dextrose is used. The temperature is 30° C. and the oscillations are 100 per minute.

Natural crystalline vitamin B_1 mg.	Cc. of gas in 3 hours.
none	185
0.001	215
0.005	305
0.010	350
0.040	395
0.100	405

This method agrees with estimations of vitamin B_1 made with the rat-growth method.

Formaldehyde-Azo Test—Vitamin B_1 having a pyrimidine ring will couple with a diazo compound under controlled conditions.

[11] Prebluda and McCollum, *Science* **84**, 448 (1936).

[12] Schultz, Atkin, and Frey, *J. Am. Chem. Soc.* **59**, 948, 2457 (1937).

Kennersley and Peters[13] found that the test for the vitamin with diazotized sulfanilic acid requires a definite pH and is more stable in the presence of formaldehyde.

Reagents: a) Sulfanilic acid solution—Dissolve 4.5 g. of sulfanilic acid and 45 cc. of hydrochloric acid (sp. gr. 1.19) in water and make up to 500 cc.

b) Sodium nitrite solution—Dissolve 25 g. of 90 per cent sodium nitrite in water and make up to 500 cc.

Add 1.5 cc. of solution (a) to 1.5 cc. of solution (b) and then the mixture is left on ice for 5 minutes. Six cc. more of (b) is added and the mixture is again placed on ice for 5 minutes. Dilute to 50 cc. and replace on ice for 15 minutes before use. This solution is the diazotized sulfanilic acid.

c) Dissolve 5.76 g. of sodium bicarbonate in water and make up to 100 cc. Add 100 cc. of 1 *N* sodium hydroxide solution.

Determination: Add 0.5 cc. of the diazotized sulfanilic acid to 1.25 cc. of the sodium bicarbonate-sodium hydroxide reagent in a small test tube. After 1 minute, 0.3 cc. of 40 per cent formaldehyde is added. Then 0.1 to 0.3 cc. of the solution of vitamin B_1 of acidity greater than pH 4.0 is added. A pink color develops slowly, and increases in intensity for 30–60 minutes, after which time it is practically constant. Comparison may be made in a colorimeter against standard vitamin treated the same way.

Vitamin C

Waugh and King[14] isolated the vitamin from lemon juice. They found that this crystalline material was identical with the hexuronic acid of Szent-Gyorgyi.[15] The chief property of ascorbic acid and undoubtedly this is a chemical property, although the exact nature of its action is unknown, is the antiscorbutic action of this compound. It is a reducing substance and reduces Fehling solution, neutral silver nitrate and neutral potassium permanganate. It is easily oxidized by iodine and Benedict solution as well as by atmospheric oxygen in alkaline solution. This property is the basis of most of the methods for the estimation of the vitamin. Vitamin C has been synthesized by a number of investigators.

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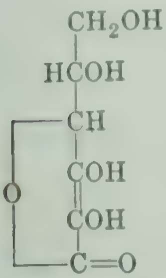
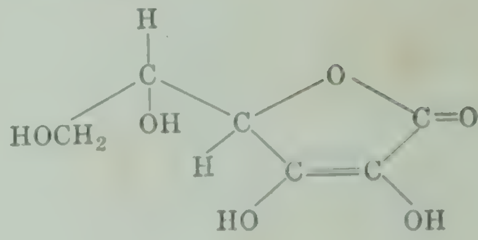
[13] Kennersley and Peters, *Biochem. J.* 28, 667 (1934).
[14] Waugh and King, *J. Biol. Chem.* 97, 325 (1932).
[15] Szent-Gyorgyi, *Nature* 129, 943 (1932).

The carbon, hydrogen combustion indicates a formula of $C_6H_8O_6$. Haworth[16] presents the following formula



l–Ascorbic acid



Tillmans[17] noted the reducing power of some unknown substance, later identified as ascorbic acid, vitamin C, in natural foods and used the oxidation-reduction indicator 2,6 dichlorophenolindophenol, sodium 2,6 dichlorobenzenoneindophenol, to distinguish between natural juices and artificial juices and later pointed out that there was a close degree of correlation between the reducing capacity of these juices and their antiscorbutic activity. This correlation was confirmed by many other investigators.

Preparation of Sample—In substances from which the juice may easily be extracted by mechanical pressure and subsequent centrifuging, such as oranges, lemons, limes and other citrus fruits, the resultant clear juice may be used directly for the determination. Other food materials such as milk may be treated as described in Chapter VI, section, "Reconstituted Milk," that is preparing a trichloroacetic acid serum. Solid materials may be ground and mixed with sand and then extracted with trichloroacetic acid solution and subsequently diluted so that the final concentration of the trichloroacetic acid is about 5 per cent.

Iodine Titration Method—This method is especially applicable to fruit and tomato juices and is a simple and rapid method for the differentiation of true or natural juices and artificial juices. Cut the fruit in half and extract the juice with a hand orange squeezer. Centrifuge and filter through a large size Gooch crucible fitted with a Fisher cotton filter pad. Pipette 5 cc. of the juice or the prepared solution and transfer to a 125 cc. flask. Add 20 cc. of water and 2 cc. of a 1 per cent soluble starch solution. Titrate rapidly with an accurately standardized 0.01 *N*

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[16] Haworth, *Nature* 129, 576 (1932).

[17] Tillmans, Hirsch and Reinhagen, *Z. Untersuch. Lebensm.* 56, 272 (1928).

iodine solution containing 16 g. potassium iodide per liter. Each cc. of iodine is equivalent [18] to 0.88 mg. of ascorbic acid, lactone form. The mg. of vitamin C per cc. can be calculated from this relationship. The titration must be rapidly performed because other substances such as glutathione and cysteine are oxidized slowly by iodine solution, consequently rapid titration minimizes this possible error.

When juices with a low vitamin C content or suspected artificial juices or fruit drinks with a small proportion of natural fruit juices are analyzed, 25 cc. of the original material may be taken for the analysis and no additional water need be added.

Dye Method—Weigh accurately 125 mg. of sodium 2,6 dichlorobenzenoneindophenol, dissolve in small portions of warm water and filter. The filtered solution, when cool, is made up to 250 cc. The indicator solution keeps better if prepared in a 7.2 pH phosphate buffer. The strength of the dye solution may be estimated in three ways. 1) Five cc. of the dye is transferred to a 50 cc. flask and diluted with 20 cc. of water and a few drops of acetic acid. This is titrated with lemon juice prepared as directed above, with the aid of a microburette or semi-microburette until the dye is decolorized. The color of the dye changes from blue to red to colorless. The blue is the neutral or alkaline color and the red is the acid color. Then 5 cc. of the lemon juice is titrated with the standard 0.01 *N* iodine solution. Since each cc. of 0.01 *N* iodine solution used is equivalent to 0.88 mg. of ascorbic acid, then the number of cc. of lemon juice multiplied by the ascorbic acid content as derived by the iodine titration and divided by 5 yields the value of ascorbic acid in mg. per cc. of dye solution.

2) Titrate 25 cc. of dye solution with a 0.01 *N* solution of ascorbic acid made by dissolving 276 mg. ascorbic acid (Merck's cebione) in water and making up to 100 cc. volume. The equivalent value of each cc. of dye solution is thus determined directly.

3) Pipette 15 cc. of the dye solution into a small flask. Add 0.5 to 1.0 g. of potassium iodide and 0.5 to 1.0 cc. sulfuric acid (1:4). Shake to facilitate oxidation and titrate the liberated iodine with 0.01 *N* sodium thiosulfate solution using starch solution as indicator. One cc. of 0.01 *N* thiosulfate solution is equivalent to 0.88 mg. ascorbic acid.[18a,b]

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[18] Bessy and King, *J. Biol. Chem.* 103, 687 (1933).

[18a] Buck and Ritchie, *Ind. Eng. Chem., Anal. Ed.* 10, 26 (1938).

[18b] Menaker and Guerrant, *Ind. Eng. Chem., Anal. Ed.* 10, 25 (1938).

Determination: Five cc. of the dye solution is measured by means of a pipette into a 125 cc. flask and 20 cc. of water is added. This is titrated with the prepared juice from a microburette or semi-microburette. Five cc. of the dye generally is equivalent to 1.06 mg. of ascorbic acid. Divide this factor or the appropriate factor by the number of cc. of juice required to decolorize 5 cc. of the dye solution. The result is the number of mg. vitamin C per cc. of juice. This method is modified from that of Birch, Harris and Ray.[19]

Stability of Vitamin C

It has been the general impression that the vitamin C content of orange juice, other juices and milk degrades rapidly. The work of various investigators shows that the vitamin content of these food materials when kept under moderate refrigeration decreases slowly. Nelson and Mottern [20] kept orange juice hermetically sealed under atmospheres of oxygen, nitrogen and air and frozen for a year. They found that there was no appreciable loss of vitamin C content at the end of that time. Other investigations show that heating foodstuffs does not destroy all of the vitamin C content. Thus pasteurized milk has less vitamin C and correspondingly less antiscorbutic activity than raw milk but the vitamin is not completely destroyed.

The vitamin C present in milk and other materials can be regenerated by the addition of hydrogen sulfide.[21] The hydrogen sulfide may then be removed by passing a stream of nitrogen through the solution until no test for hydrogen sulfide is obtained in the exhaust gas. The titration for the vitamin may then be performed in the usual manner. This regeneration is of value, for investigators have shown that the body can do it also.

Orange Drink

There are three main reasons for the purchase of orange drink. First, in order to quench thirst, secondly, to obtain the benefits of a fruit drink containing vitamin C and thirdly, to build up the alkaline reserve through the ash content of the fruit juice. The second and third are the only reasons when one orders orange drink with a meal, as for example, breakfast or a rapid lunch. The layman has been educated in the importance

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[19] Birch, Harris and Ray, *Biochem. J.* 27, 590 (1933).

[20] Nelson and Mottern, *Ind. Eng. Chem.* 25, 216 (1933).

[21] Johnson, *Biochem. J.* 27, 1287 (1933).

of having vitamin rich foods. In many cases, it becomes necessary for him to pay particular attention to the quantity of vitamin he ingests in order to combat some disorder. This person may be mislead by some flagrantly adulterated and misbranded orange drink.

The quantity of orange juice in an orange drink may be estimated by analyses of total solids, ash, alcohol precipitate, pectin, pectic acid, acid and protein. However, a much shorter means may be used, if it is borne in mind that the vitamin C content does not degrade rapidly when the orange drink is stored under proper conditions. This method is one based on the average vitamin C content of oranges. The average vitamin C content or ascorbic acid content of orange juice is 0.5 mg. per cc.

Determine the number of mg. of ascorbic acid per cc. in the orange drink or orangeade and divide by the factor 0.5 as defined above. The quotient multiplied by 100 yields the per cent of orange drink in the product, based of course, on the above mentioned average vitamin C content.

The same procedure may be developed for other fruit drinks and for tomato juice.

Vitamin D

The work of Windaus, Waddell, Bills, and others has shown that vitamin D is not a single substance. The term vitamin D designates the entire group of substances which are antirachitically active. Bills [22] states that eight forms of vitamin D have been artificially prepared and at least two forms occur in fish oils which may or may not be identical with certain of those made synthetically.

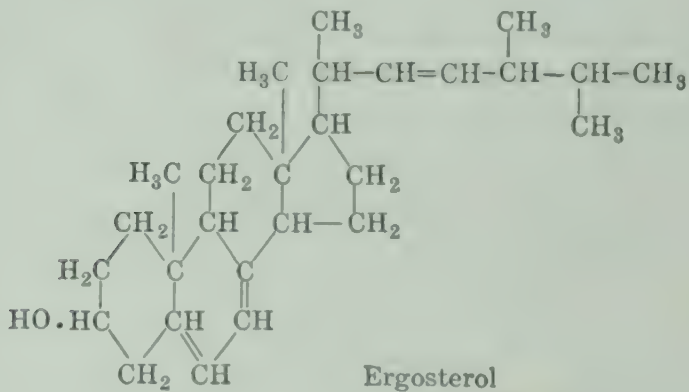
The structure of ergosterol, $C_{28}H_{44}O$, is established with a high degree of certainty because the chemistry of this sterol has been explored with great interest on account of its relationship to vitamin D. Calciferol is the antirachitic compound formed by the photochemical transformation of ergosterol with ultraviolet light. It is an isomer of ergosterol, the parent or provitamin substance. Calciferol has never been isolated from a natural vitamin D containing food. Viosterol is irradiated ergosterol and is composed of calciferol and inactive sterol photo-decomposition products dissolved in oil. It is effective in the cure and prevention of rickets.

Other irradiatea sterols are capable of producing substances having vitamin D properties although they are much less potent than calciferol

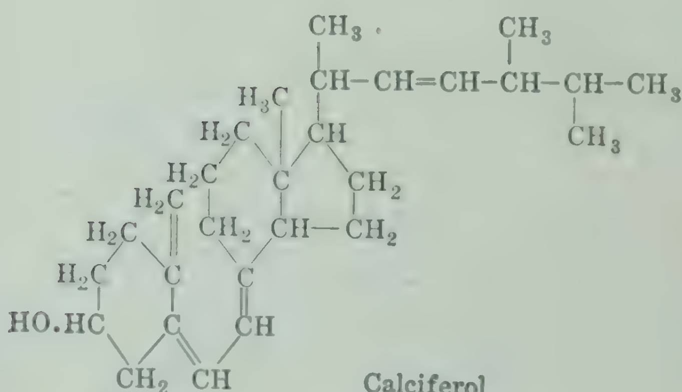
[22] Bills, *J. Am. Med. Assoc.* **108**, 13 (1937).

when assayed by the rat test method. Pure cholesterol possesses a provitamin and certain derivatives of cholesterol are probably provitamin D substances for by irradiation cholesterol types of vitamin D are produced as distinguished from the ergosterol type of vitamin D.

One form of natural vitamin D has been isolated as an ester. Brockmann[23] isolated the 3,5 dinitrobenzoate of vitamin D of tuna liver oil which he found identical with the vitamin D_3 ester produced by irradiation of 7-dehydrocholesterol. Vitamin D_3 has a potency of 25,000 International units per mg. while calciferol or vitamin D_2 has a potency of 40,000 International units per mg.



Ergosterol



Calciferol

Until 1934 the idea prevailed that ultraviolet energy of sunlight activated minute amounts of ergosterol, a plant sterol, which was held to be

[23] Brockmann, *Z. physiol. Chem.* 241, 104 (1936).

[24] Anderson, Bacharach and Smith, *Analyst* 62, 430 (1937).

the main precursor of vitamin D activity in the body. However, Waddell's experiments with cholesterol shows this view is no longer tenable. Instead, the antirachitic effect of exposure to sunlight can be explained by the irradiation of naturally occurring cholesterol, containing provitamin D, in the subcutaneous fatty skin tissue. Waddell clearly shows that the provitamin D of ordinary cholesterol is not ergosterol and that the vitamin D produced by irradiating ordinary cholesterol is not calciferol.

Antimony Trichloride Reaction of Vitamin D[25]—Vitamins D_2 and D_3 give with antimony trichloride in chloroform an orange yellow color which soon reaches maximum intensity and shows a sharp absorption band at 500 mμ. Tachysterol behaves similarly. None of the other sterols, nor vitamin A have this sharp absorption band, hence this absorption may be used to estimate the vitamins. Place 0.2 cc. of the solution to be tested in the absorption cell and add 4 cc. of a saturated solution of antimony trichloride in dry chloroform. After 10 to 15 minutes the extinction coefficient is measured and the vitamin concentration may be read from a calibration curve.

Tzoni Method[26]—This method is based on the production of a red-violet color when vitamin D, pyrogallol and aluminium chloride react. If the vitamin is in a pure form and dissolved in absolute alcohol, benzene, petroleum ether, or chloroform or other suitable solvent, it may be estimated directly. Otherwise, for example, if it is dissolved in oil or fat, it must be separated by prior saponification and subsequent extraction. The oil or fat is saponified and the soap is extracted 4 times with petroleum ether. The petroleum ether extract is washed with a solution of one part of alcohol and one part 10 per cent sodium chloride solution. The wash solution is washed in turn with another portion of petroleum ether. The combined petroleum ether layers are dried with anhydrous sodium sulfate and the ether solution is then distilled in vacuum in an atmosphere of carbondioxide. The residue is dried in vacuum over sulfuric acid after which it may be dissolved in one of the dry solvents mentioned above.

If the vitamin is mixed with other sterols in moderate proportions no interference results. However, if large quantities of other sterols are present, they should be eliminated by precipitation with 1 per cent solu-

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25 Brockmann and Chen, *Z. physiol. Chem.* **241,** 129 (1936).

26 Tzoni, *Biochem. Z.* **287,** 18 (1936).

tion of digitonin in alcohol. The precipitate is filtered off and is washed with a solution of 73 parts of acetone, 18 parts of water and 9 parts of absolute alcohol. The filtrate is mixed with an equal volume of 10 per cent sodium chloride solution and the vitamin is extracted with petroleum ether as directed above.

Vitamin A, carotene and other substances that react with the reagents must be eliminated also.

Reagents: a) Pyrogallol solution.—0.1 per cent solution of pure pyrogallol in absolute alcohol. This solution is kept in a dropping bottle.

b) Aluminium chloride solution. Place as rapidly as possible large pieces of anhydrous aluminium chloride in an Erlenmeyer flask and add sufficient absolute alcohol to obtain a 10 per cent solution. Stopper the flask with a stopper fitted with a calcium chloride tube and then shake the flask carefully until all the aluminium chloride is dissolved. Filter the solution rapidly into a dropping bottle and stopper securely.

Determination: Measure a portion of the vitamin solution, not to exceed 2 cc. into a dry clean test tube and add 5 drops of the pyrogallol solution. Evaporate to about 0.1 cc. Add 3 drops of the aluminium chloride solution and again heat in a water bath. The presence of vitamin D is indicated by the rapid development of a red-violet crust. On solution in absolute alcohol, a lilac-red to red-violet color develops.

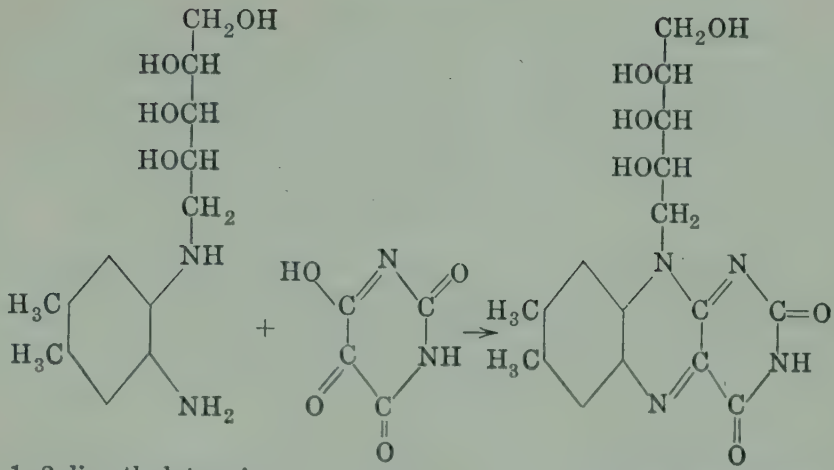
This color may be matched against a mixture of 1 cc. of a solution of 0.01 g. of acid fuchsin in 1 liter of water and 2 cc. of a solution of 0.09 g. of Renol black in 1 liter of water, diluted with 20 cc. of water.

It is necessary, for proper procedure, that all the glassware and the reagents be dry and that the pyrogallol be added prior to the addition of aluminium chloride for aluminium chloride also reacts alone with the vitamin.

Vitamin B_2

As was stated in the opening section of this chapter, vitamin B_2 is not synonymous with vitamin G, but is one of the factors composing vitamin G. Vitamin B_2 has been identified as lactoflavin. It is a growth promoting factor. It has been synthesized by Kuhn and coworkers[27] from 1,2-dimethyl-4-amino-5[d-1^1-ribitylamino] benzol and alloxan, which reacts in the lactim form to yield 6,7-dimethyl-9-d-riboflavin (6,7-dimethyl-9-d-1-ribitylisoalloxazine), that is lactoflavin.

[27] Kuhn, Reinemund, Wegand and Strobele, *Ber.* 68, 1765 (1935).



1, 2-dimethyl-4-amino 5 [*d-l*-ribitylamino] benzol — alloxan — 6, 7-dimethyl-9-*d*-riboflavin (Lactoflavin)

Vitamin B_2 may be estimated fluorometrically.[28] It may be obtained by adsorption on fullers earth or frankonite from fresh liver extracts or other lactoflavin bearing extracts in neutral or strongly acid solutions. The adsorption is generally complete in 10 minutes. The adsorbates may be eluted with diluted 10–50 per cent aqueous diethylamine or 0.2 per cent sodium hydroxide solution. The amount of lactoflavin is then determined by estimation of the fluorescence.

Rapid Method for the Determination of Lactoflavin in Milk—It was observed by Whitnah, Kunerth and Kramer [29] that the trichloroacetic acid serum from milk used for vitamin C titrations often had a greenish color whereas the mercuric nitrate serum used for the determination of sugar was colorless. Based on this fact these investigators developed the following procedure for the fluorimetric estimation of flavin in milk.

Add 15 cc. of 10 per cent trichloroacetic acid to 10 cc. of milk, let stand thirty to sixty minutes, centrifuge for 5 minutes. Neutralize 10 cc. of the resulting serum, using methyl orange as the indicator, and dilute until the sample can be matched in the light of a lamp,[30] with standard flavin solution containing 0.12 to 0.06 gamma of flavin per cc. Calculate

[28] Lepkovsky, Popper and Evans, *J. Biol. Chem.* **108**, 257 (1935).
[29] Whitnah, Kunerth and Kramer, *J. Am. Chem. Soc.* **59**, 1153 (1937).
[30] Eveready Fluoray lamp.

the flavin content on the basis of dilutions made. Dilutions, until the portions read, contain less than 0.12 gamma per cc., seem essential as the values for stronger solutions are easily underestimated.

Vitamin Units

Owing to the confusion which has arisen from the employment by various workers of different standards for measuring and expressing vitamin potencies, the Commission on Biological Standardization of the League of Nations has recommended the following vitamin units for international usage.

1) Vitamin A unit = The vitamin A activity of 0.6 microgram (0.6 gamma) of pure β-carotene prepared from carrots by Willstätter's method.

2) Vitamin B_1 unit = The antineuritic activity of 10 mg. of a standard adsorption product of the vitamin prepared from an extract of rice polishings. Crystalline vitamin B_1 is now available and has been evaluated in terms of the international unit. The values vary from 2 to 5 micrograms (2 to 5 gammas) for one international unit depending upon the method of assay. Four gammas is probably nearer the true ratio.[31]

3) Vitamin C unit = The vitamin C activity of 0.05 mg. l-ascorbic acid.

4) Vitamin D unit = The vitamin D activity of 1 mg. of the international standardized solution of irradiated ergosterol, which has been found equal to that of 0.025 micrograms (0.025 gamma) of crystalline vitamin D (calciferol).

Vitamin Equivalent Units—1.4 international units = 1 Sherman or 1 U. S. P. unit.

Sherman B_1 and Steenbock D units are based on the response of animals and as the value of the units depends on the strain of rats used, the conversion factors into international units are different for different laboratories. Thus the conversion factor of vitamin B_1 varies from 1.3 Sherman units to 1 international unit to 4 Sherman units to 1 international units.

The accepted international conversion ratio for vitamin D is 2.7 international units = 1 Steenbock unit.

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[31] Light, personal communication (1937).

REFERENCES

Harris, "Vitamins," Macmillan (1935).
Sherman, "Chemistry of Food and Nutrition," 5th ed., Macmillan (1937).
United States Pharmacopeia XI, Decennial Revision.
Annual Review of Biochemistry, Vol. 5 (1936).
Best and Taylor, "Physiological Basis of Medical Practice," Wood (1937).

CHAPTER XVII

INORGANIC DETERMINATIONS

THERE is little reason to explain the necessity of this chapter in a book on food analysis. The importance of the inorganic constituents in foods is too well recognized. In this chapter the methods described are generally specific. As the author has mentioned previously, methods which do not have specificity are to be regarded with caution and must be followed in great detail after the elimination of interferences before definite conclusions can be drawn.

FLUORINE

The determination of fluorine as developed by Willard and Winter [1] is based on the isolation of fluorine accurately and expeditiously from interfering materials by distillation as hydrofluosilicic acid which may subsequently be estimated colorimetrically by the bleaching of a zirconium-alizarin lake or by titration with thorium or cerous nitrate. These estimations are of importance because fluorine causes mottling and discoloration of teeth as well as being a poison.

The estimation of small amounts of fluorine is difficult for unless the proper fixative is used, the fluorine will be lost in the ashing or will not be completely volatilized when the ash is distilled with perchloric acid. Winter [2] recommends magnesium acetate as the most satisfactory fixative in the following method.

Place 5–25 g. of material, according to the fluorine content, in a crucible or porcelain dish, add sufficient 5 per cent magnesium acetate solution to moisten completely and no more. Dry in an oven for at least 24 hours, and ash in a muffle at dull redness. Brush the ash into the distillation flask.

The distillation apparatus, Fig. 54, consists of a Claissen flask with necks 10 cm. long instead of the usual length. The side arm that connects the flask with the condenser, is bent upward for about 4 cm. and

[1] Willard and Winter, *Ind. Eng. Chem., Anal. Ed.* 5, 7 (1933).

[2] Winter, *J. Assoc. Official Agr. Chem.* 19, 362 (1936).

then downward at two points in order to fit a vertical condenser. Preferably the side arm should fit the condenser by a ground glass joint. More elaborate trapping devices are inadvisable, because the possible adsorption causes fluorine deficiencies. The straight neck of the distilling flask carries a rubber stopper fitted with a thermometer and a dropping funnel

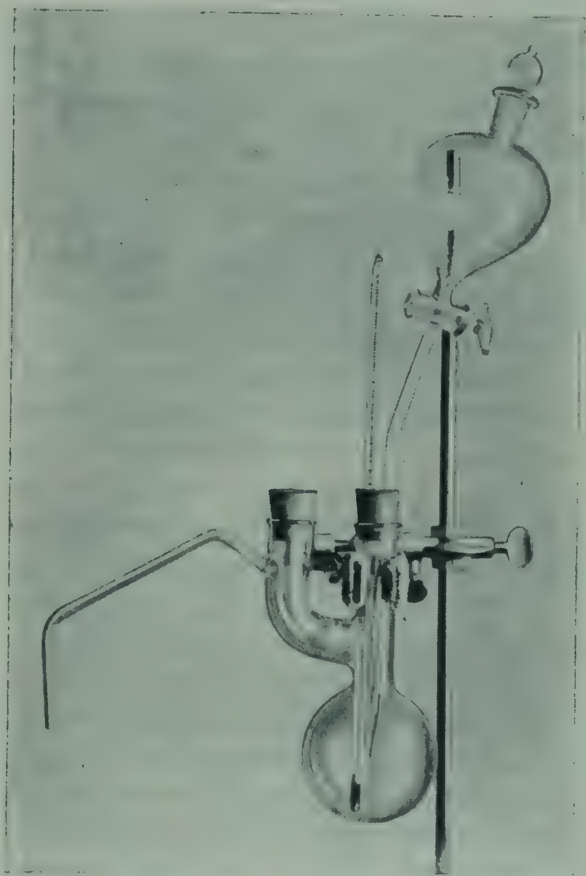

Fig. 54. Fluorine Distillation Apparatus

whose stem has been drawn to a capillary. Both the thermometer and the dropping funnel extend to within 5 mm. of the flask bottom.

Wash the crucible or dish several times with water and a small amount of perchloric acid or sulfuric acid, adding the wash solution to the flask. Connect the apparatus as directed above. Remove the stopper, add perchloric acid or sulfuric acid slowly from a pipette until the effervescence ceases and then add approximately 15 cc. more of perchloric acid or 12 cc. more of sulfuric acid. Replace the stopper, boil at 135–140° C. and

collect the distillate in a 100 cc. volumetric flask. When the liquid temperature reaches 135° C. sufficient water is slowly dropped from the funnel to compensate for the water distilling out and to maintain the temperature at 135° C. The distillation requires constant supervision. After the 100 cc. flask is filled (distillate 1) collect another 50 cc. (distillate 2) to be certain that all the fluorine has been volatilized.

Colorimetric Method—Dissolve 0.87 g. of zirconium nitrate, $Zr(NO_3)_4.5H_2O$, in 100 cc. of water, and 0.17 g. of sodium alizarinate in 100 g. of water. Mix equal parts of the two solutions and dilute the mixture (1:4) with water.

Make up a series of standards in Nessler tubes or test tubes of about 80 cc. capacity by placing 0.02, 0.04, 0.06, 0.09, 1.20 mg. of fluorine in each tube, respectively. Add water to make about 50 cc., 10 cc. of hydrochloric acid (1:1), mix thoroughly, add 2 cc. of the dye solution and bring all the tubes to the same level with water. Again mix thoroughly, place the tubes in a steam bath for 30 minutes, and cool. For unknowns take aliquots of the distillates whose fluorine contents fall within the above range of standards. The fluorine is determined from the nearest standard.

The fading of the zirconium-alizarine lake is a measure of the amount of fluorine present, a procedure developed by Smith and Dutcher.[3] If quantities of fluorine fall below the range given, less dye should be used and if quantities above the range are to be determined more dye should be used.

The individual tubes show a fading in color, which increases as the fluorine content increases, hence a comparison of the fading caused by an aliquot of the unknown with the standards prepared gives the measure of the fluorine content of the sample.

Titration Method[4]—Several drops of 0.04 per cent phenol red solution are added to the distillate obtained as detailed in the foregoing and the liquid is neutralized with dilute sodium hydroxide, avoiding a large excess. The alkaline solution is boiled and repeatedly brought back to the apparent neutral point with 0.02 *N* or 0.01 *N* perchloric acid. During this neutralization, the volume is reduced to 5 to 10 cc. When the faint pink color is no longer restored by boiling (carbonate free), the solution is cooled, transferred quantitatively to a 50 cc. beaker, and boiled from about

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[3] Smith and Dutcher, *Ind. Eng. Chem., Anal. Ed.* **6**, 61 (1934).

[4] Scott and Henne, *Ind. Eng. Chem., Anal. Ed.* **7**, 299 (1935).

25 cc. down to 2 to 3 cc. Two drops of a saturated alcoholic solution of methyl red and 10 drops of 0.04 per cent bromocresol green solution are added. The bromocresol green is weighed out exactly and neutralized with standard sodium hydroxide to yield the monosodium salt. This prevents alteration of the neutrality of the solution when the indicator is added. The liquid is titrated at 80° C. to the maximum red color with cerous nitrate solution, 1 cc. of which is equivalent to 0.5 mg. of fluorine. When the amount of fluorine is less than 0.2 mg., thorium nitrate solution, 1 cc. of which is equivalent to 0.1 mg. of fluorine, may be substituted for the cerous nitrate, using the same mixed indicator.

Churchill, Bridges and Rowley [5] point out that phosphates may interfere in this determination, for in some food products the phosphates are possibly reduced to a form which is readily carried over in the distillate. They recommend a double Willard-Winter distillation of the fluorine from the ash of foods. The first should be made with sulfuric acid to eliminate hazard, as some carbonaceous material may be present, and the second distillation should be made with perchloric acid at 135° C. This procedure yields a distillate free from sulfate and phosphate.

Selenium

Selenium traced to vegetation grown in soil areas that contained selenium caused illness in animals and may very likely cause illness in humans. Thus Munsell, DeVaney, and Kennedy [6] measured the toxicity of food containing selenium by its effect on rats. Because of the possible danger to humans, rapid and accurate methods are necessary. The following method developed by Robinson, Dudley, Williams and Byers [7] is based on the fact that selenium may be separated from all other elements except arsenic and germanium by distillation with concentrated hydrobromic acid. The selenium must be in, or converted into, the hexavalent condition before distillation in order to insure its distillation with the acid will be complete. In most cases the conversion may be accomplished by the use of bromine. The excess of bromine distills at a low temperature and the hydrobromic acid then reduces the selenium to the quadrivalent condition. In this form it readily distills along with the hydrobromic acid. The selenium is subsequently estimated in the dis-

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[5] Churchill, Bridges and Rowley, *Ind. Eng. Chem., Anal. Ed.* 9, 222 (1937).

[6] Munsell, DeVaney and Kennedy, U. S. Dept. of Agr., Tech. Bull. No. 534 (1936).

[7] Robinson, Dudley, Williams and Byers, *Ind. Eng. Chem., Anal. Ed.* 6, 274 (1934).

tillate by reduction with hydroxylamine hydrochloride and sulfur dioxide.

Vegetable Matter: Stir 100 g. of the well ground and mixed vegetation into a concentrated solution of 25 g. of magnesium nitrate, and add 5 g. of magnesium oxide. Dry the mass over a water bath and finally in an oven at 105° C. Ignite the dried material slowly in a muffle until the ash is a uniform gray color. After ignition, triturate the ash with 100 cc. of concentrated hydrobromic acid, capable of being completely decolorized with sulfur dioxide, and 2 cc. of bromine, transfer to a distilling flask and estimate as detailed below.

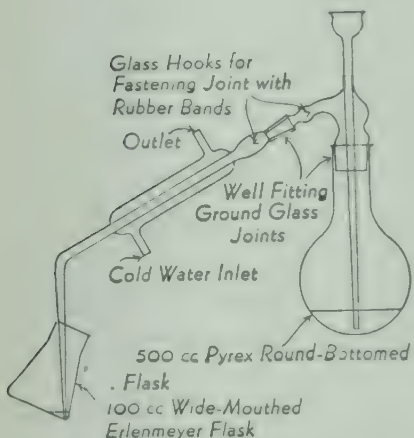

FIG. 55. Selenium Distillation Apparatus

Animal Matter:[8] The material in a suitable state of subdivision is placed in a beaker of 400 to 600 cc. capacity, covered with 150 to 200 cc. of nitric acid, and allowed to stand at room temperature for from 2 to 3 hours, during which period it is stirred vigorously at intervals. Fifty cc. of hydrogen peroxide, 30 per cent by weight, is added and the mixture is allowed to stand overnight. If frothing occurs on the addition of the hydrogen peroxide, foaming over is prevented by vigorous stirring of the foam. After standing overnight, the mixture is warmed slowly on the steam bath until frothing ceases, after which 50 cc. more of hydrogen peroxide is added, together with 20 cc. of sulfuric acid. The mixture is then taken to essentially complete dryness on the steam bath or hot plate. The cooled black paste is treated with 100 cc. of concentrated hydrobromic acid to which has been added sufficient bromine to make it deep yellow in color. The material is then transferred to the distillation flask.

The apparatus, Fig. 55, consists of a Pyrex 500 cc. round bottom flask fitted with a ground glass stopper into which has been sealed a thistle tube with a stem long enough to reach within 5 mm. of the bottom of the flask. The ground glass stopper also has a side arm with a ground glass end fitted to a condenser whose end is drawn out into a long adapter, bent and with a capillary tip so that it may fit easily into a 100 cc. wide mouth Erlenmeyer flask, which acts as the receiver.

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[8] Dudley and Byers, *Ind. Eng. Chem., Anal. Ed.* 7, 3 (1935).

Connect the distillation apparatus described with the adapter just below the surface of 2 to 3 cc. of bromine water in the receiver flask and apply heat gradually. One or two g. of bromine should distill over in the first few cubic centimeters of distillate. If insufficient bromine has been added to produce this quantity of bromine, more must be added through the thistle tube. A somewhat greater excess of bromine does no harm, but too great an excess is to be avoided because of the formation of too much sulfuric acid later. Collect 30 to 50 cc. of the distillate by increasing the heat. Make a second, or even third distillation with intervening additions of hydrobromic acid and bromine through the thistle tube, unless it is certain from experience that all the selenium is in the first distillate. Remove the distillate and pass in sulfur dioxide until the yellow color due to bromine is discharged. Add 0.25 to 0.5 g. of hydroxylamine hydrochloride, stopper the flask loosely, put on the steam bath for an hour, and allow to stand overnight at room temperature. If selenium is present it will appear as a characteristic pink or red precipitate. If much selenium is present it will shortly turn black.

Collect the precipitated selenium on an asbestos pad in a porcelain crucible, and wash slightly with hydrobromic acid containing a little hydroxylamine hydrochloride. Dissolve the selenium on the pad by passing through 10 to 15 cc. of a solution of 1 cc. of bromine in 10 cc. of hydrobromic acid in small quantities and wash into a 25 cc. measuring flask if the quantity is small and is to be estimated colorimetrically. If over 0.5 mg., filter into a small beaker, precipitate as before, gather on an asbestos pad as before, and wash with hydrobromic acid containing a little hydroxylamine hydrochloride and then with water. Prepare a tare in the same way. Dry at 90° C. for 1 hour, place in a vacuum desiccator, and exhaust the air while the crucibles are still hot. Cool 0.5 hour. Allow the air to enter the desiccator, cool an additional 0.5 hour, and weigh against the tare. Check the weight by drying again. If the quantity is small and is to be estimated colorimetrically, add 1 cc. of a solution containing 5 per cent gum arabic and precipitate the selenium by sulfur dioxide and hydroxylamine hydrochloride. Prepare comparison solutions containing known quantities of selenium in exactly the same manner and allow them to stand overnight. Shake the standard and test solutions and compare the depth of color in Nessler tubes. This comparison is best carried out in sunlight. It is difficult to match solutions containing more than 0.5 mg. of selenium in 25 cc. and the color comparison is most satisfactory when 0.01 to 0.1 mg. is present.

Williams and Lakin[9] recommend the following procedure for preparation of the sample prior to the selenium distillation. To prepare a sample of air-dry vegetation, it is first ground to pass a 2 mm. mesh sieve, then mixed and quartered. A weighed sample usually 10 g. is stirred into a mixture of 50 cc. of sulfuric acid and 100 cc. nitric acid in a 600 cc. Pyrex beaker. The mixture is stirred with a thermometer until it becomes homogeneous, after the first few minutes with gentle heating, without allowing the temperature to rise above 100° C. After all frothing has ceased, the temperature of the mixture is raised to a maximum of 120° C. until all evolution of nitrogen peroxide has ceased. The end of the operation is marked also by an incipient carbonization of the mixture, though longer heating at 120° C. does little harm. After the mixture is cooled it is transferred to the all glass distilling flask, described above, 100 cc. of hydrobromic acid and 1 cc. of bromine are added and 75 cc. of the distillate is collected. Care, of course, must be taken that the first portion of the distillate contains a small excess of bromine.

Phosphorus

It is unnecessary to explain the importance of phosphates in food and foodstuffs. Suffice it to say that in food materials phosphorus is usually determined and estimated as phosphoric acid expressed as P_2O_5. This may be done gravimetrically as magnesium pyrophosphate, $Mg_2P_2O_7$, or volumetrically, or colorimetrically. In general, the food sample is ashed by one of the number of methods previously described, and then a suitable aliquot of the ash or the entire ash dissolved in sulfuric acid or in nitric acid or in both, or the wet ash is used.

Gravimetric Method—*Reagents:* a) Molybdate solution. Dissolve 100 g. of molybdic acid, MoO_3, in a mixture of 144 cc. of ammonium hydroxide and 271 cc. of water. Pour this solution slowly and with constant stirring into a mixture of 489 cc. of nitric acid and 1148 cc. of water. Keep the final mixture in a warm place for several days or until a portion heated to 40° C. deposits no yellow precipitate of ammonium phosphomolybdate. Decant the solution from any sediment and preserve in glass stoppered bottles.

b) Magnesia mixture. Dissolve 11 g. of magnesium oxide in hydrochloric acid (1:4), avoiding an excess of the acid; add a little magnesium

[9] Williams and Lakin, *Ind. Eng. Chem., Anal. Ed.* 7, 409 (1935).

oxide in excess; boil a few minutes to precipitate iron, aluminium, and phosphorus pentoxide, and filter. To the filtrate add 140 g. of ammonium chloride and 130.5 cc. of ammonium hydroxide and dilute to 1 liter. Or dissolve 55 g. of magnesium chloride, $MgCl_2.6H_2O$, in water, add 140 g. of ammonium chloride and dilute to 870 cc. Add ammonium hydroxide to each required portion of the solution just before using, in the proportion of 15 cc. to 100 cc. of solution.

c) Magnesium nitrate solution. Dissolve 150 g. of magnesium oxide in nitric acid (1:1), avoiding an excess of the acid; add a little magnesium oxide in excess, boil, filter from the excess of magnesium oxide, ferric oxide, etc., and dilute to 1 liter.

Determination: Transfer the solution containing the ash to a 250 cc. beaker; add ammonium hydroxide in slight excess, and barely dissolve the precipitate formed with a few drops of nitric acid, stirring vigorously. If hydrochloric acid or sulfuric acid had been used as a solvent, add about 15 g. of crystalline ammonium nitrate or a solution containing that quantity. To the hot solution add 70 cc. of the molybdate solution for every decigram of phosphorus pentoxide present. Digest at about 65° C. for 1 hour, and determine whether or not the phosphorus pentoxide has been completely precipitated by adding more molybdate solution to the clear supernatant liquid. Filter, and wash with cold water or preferably with a solution of a 100 g. of ammonium nitrate dissolved in and diluted to a liter of water. Dissolve the precipitate on the filter with ammonium hydroxide (1:1) and hot water and wash into a beaker to a volume of not more than 100 cc. Neutralize with hydrochloric acid, using litmus paper or bromthymol blue as indicator; cool; and from a burette add slowly, at about 1 drop per second, stirring vigorously, 15 cc. of the magnesia mixture for each decigram of phosphorus pentoxide present. After 15 minutes, add 12 cc. of ammonium hydroxide. Let stand until the supernatant liquid is clear (about 2 hours), filter, wash the precipitate with ammonium hydroxide (1:9) until the washings are practically free of chlorides, dry, burn at a low heat, and then ignite in an electric furnace at 950–1000° C., cool in a desiccator and weigh as magnesium pyrophosphate, $Mg_2P_2O_7$. Calculate the result as percentage phosphorus pentoxide.

Volumetric Method—Add 5–10 cc. of nitric acid, depending on the manner of solution or add the equivalent in ammonium nitrate. Add ammonium hydroxide until the precipitate that forms dissolves but slowly on stirring vigorously, dilute to 75–100 cc., and adjust to a tem-

perature of 25–30° C. Add sufficient molybdate to insure complete precipitation. Five cc. of nitric acid must be added to every 100 cc. of molybdate solution which is then filtered immediately before use. Place the solution in a shaking or stirring apparatus and shake for 30 minutes at room temperature. Decant at once through a filter, and wash the precipitate twice by decantation with 25–30 cc. portions of water, agitating thoroughly and allowing to settle. Transfer the precipitate to the filter and wash with cold water until the filtrate from 2 fillings of the filter yields a pink color upon the addition of phenolphthalein and 1 drop of the standard alkali. The standard alkali is prepared by diluting 328.81 cc. of *N* alkali to 1 liter. One cc. of this solution is equivalent to 1 mg. of phosphorus pentoxide. Transfer the precipitate and filter to the beaker or precipitating vessel, dissolve the precipitate in a small excess of the standard alkali, add a few drops of phenolphthalein indicator, and titrate with standard acid. The standard acid is prepared to be equal to or 1/2 the normality of the standard alkali solution.

Colorimetric Method[10] *Reagents:* Ammonium molybdate solution —Dissolve 25 g. of ammonium molybdate in 200 cc. of water heated to 60° C. and filter. Cool and dilute with water to 1 liter. This solution then contains 2.5 g. of ammonium molybdate per 100 cc.

Sulfuric acid solution—Dilute 280 cc. of arsenic and phosphorus-free sulfuric acid to a liter with water. This is approximately a 10 *N* sulfuric acid solution.

Stannous chloride solution—Place 25 g. of stannous chloride, $SnCl_2 \cdot H_2O$, in a solution of 100 cc. of hydrochloric acid diluted to 500 cc. with water and let stand in a warm room until dissolved; then dilute to 1 liter with water. Filter if necessary. This solution may be stored in a bottle with a side opening near the bottom and arranged with a stop cock for delivering the solution in drops. The solution may be protected from the air by floating a layer of white mineral oil about 5 mm. thick on the surface.

Standard phosphate solution—Dissolve 0.2195 g. of recrystallized potassium dihydrogen phosphate, KH_2PO_4, in water and dilute to a liter. This solution contains 50 parts per million of phosphorus and is too concentrated to use directly. A second stock solution may be made by taking 50 cc. of the first stock solution and diluting to 500 cc. The standard solution for color comparison is made by diluting 5 cc. of the second stock

[10] Truog and Meyer, *Ind. Eng. Chem., Anal. Ed.* 1, 136 (1929).

solution to 91 cc. with water; 4 cc. each of the ammonium molybdate solution and sulfuric acid solution are added and mixed thoroughly by swirling in a 150 cc. Erlenmeyer flask. Six drops of the stannous chloride solution is added and the solution is swirled again. The solution is diluted to 100 cc. and again mixed by shaking in the Erlenmeyer flask. The standard phosphate solution is ready for use although it is necessary to add a drop of stannous chloride solution every 10–12 minutes to obtain full color. One cc. of this standard phosphate solution contains 0.00025 mg. phosphorus per cc.

Determination: The ash is dissolved in 1 cc. of 10 *N* sulfuric acid. Sufficient water is added to insure complete solution and the solution is transferred to a small beaker. It is neutralized with ammonia using phenolphthalein as indicator. The solution is then transferred to a 100 cc. volumetric flask and made to volume.

An appropriate aliquot is transferred by means of a pipette to a 100 cc. volumetric flask with a mark at 91 cc., and is diluted with water to that volume. Four cc. each of the ammonium molybdate and 10 *N* sulfuric acid solutions is added, swirling after each addition. Add 6 drops of stannous chloride solution, shake and make up to volume. Compare in a colorimeter within 10 minutes with a standard prepared as directed above.

Comparison may also be made in Nessler tubes by using varying proportions of the standard phosphorus solution.

Sulfates

In general sulfates are estimated by precipitation with barium chloride solution in hydrochloric acid solution. However, a direct titration method has been developed by Schroeder.[11] This method is based on the use of the specific indicator tetrahydroxyquinone for barium in the titration of sulfate. The tetrahydroxyquinone is used as an internal indicator. Sheen and Kahler[12] recommend the following details.

Reagents: Standard barium chloride solution, the strength varying from 1 cc. = 1 mg. sulfate to 1 cc. = 50 mg. of sulfate standardized gravimetrically. An indicator composed of disodium tetrahydroxyquinone ground with dried potassium chloride in a 1 to 300 ratio, and passing a 100-mesh screen. Ethyl alcohol or alcohol denatured by formula No. 30

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[11] Schroeder, *Ind. Eng. Chem., Anal. Ed.* **5**, 403 (1933).

[12] Sheen and Kahler, *Ind. Eng. Chem., Anal. Ed.* **8**, 127 (1936).

or No. 3-A or isopropyl alcohol. Phenolphthalein indicator and bromocresol green indicator, if phosphates are present.

Procedure A. Carefully neutralize a 25 cc. sample containing up to approximately 2000 p. p. m. of sulfate with approximately 0.02 *N* hydrochloric acid until just acid to phenolphthalein. The temperature of the solution should be below 35° C. and it is advisable to work between 20–25° C. Add either 25 cc. of ethyl alcohol or one of the other solvents. Introduce the tetrahydroxyquinone, using 0.1 g. of the indicator for sulfate up to 100 p. p. m. and 0.2 g. for sulfate up to 2000 p. p. m. Swirl the flask to dissolve the indicator; the solution will be colored a deep yellow. Titrate with standard barium chloride solution, the strength to be employed depending on the approximate sulfate content of the sample. Add the standard barium chloride solution at a steady dropping rate with constant swirling of the flask until the yellow color changes to a rose. The rose color is the end point and is due to the appearance of the red barium salt of tetrahydroxyquinone. The rose color should appear throughout the body of the solution and not as spots of color.

Procedure B. (sulfate range from 2000 to 30,000 p. p. m.) Add sodium chloride according to the Table 65. The procedure is the same as in A for neutralization and titration.

TABLE 65. TETRAHYDROXYQUINONE REQUIRED FOR VARIOUS SULFATE CONCENTRATIONS

Sulfate concentrations p.p.m.	Quantity of THQ indicator g.	Strength[13] of $BaCl_2$ solution	NaCl required g.
Up to 100[14]	0.1	1	....
100 to 1,000[14]	0.2	1	....
1,000 to 2,000	0.2	4	....
2,000 to 4,000	0.4	10	2
4,000 to 10,000	0.4	10	4
10,000 to 20,000	0.6	50	8
20,000 to 30,000	0.8	50	8

Procedure C. (with phosphate up to 60 p. p. m.) Carefully neutralize a 25 cc. filtered sample with approximately 0.02 *N* hydrochloric acid until just acid, yellow range, to bromocresol green, approximate pH 4.

[13] 1 cc. = mg. SO_4.

[14] Subtract 0.1 cc. as a blank in titration.

Follow the procedure as in A or B; no correction will be required for the phosphate ion present.

Iodide

In many countries, where people live in the interior away from sea air, endemic goitre prevails. For these regions iodized salt is recommended. However, the amount should be and is rigidly controlled in certain countries, to not less than 1 part nor more than 2 parts potassium or sodium iodide in 250,000 parts of salt. The amount of potassium or sodium iodide may be easily determined by a method based on the replacement of iodine by chlorine, or bromine. Andrew and Mandeno [15] found that bromine is better. The action goes in the following manner:

$$KI + 3Br_2 + 3H_2O \rightarrow KIO_3 + 6HBr$$

and after the excess bromine is removed, the iodine is liberated by potassium iodide in faintly acid solution:

$$5KI + KIO_3 + 6HCl \rightarrow 6KCl + 3I_2 + 3H_2O$$

Determination: Dissolve 100 g. of salt in water and make up to 500 cc. in a volumetric flask, and filter. Transfer 200 cc. of the filtrate to a 500 cc. Erlenmeyer flask and add 1 cc. of bromine water and 2 cc. of *N* hydrochloric acid. Add a few pieces of pumice to prevent bumping and boil gently until the salt begins to precipitate. Dissolve the precipitate in water and cool. Neutralize with *N* sodium hydroxide solution. Make acid to methyl orange, add 0.2 g. of potassium iodide, 1 cc. of starch solution and titrate with 0.1 *N* sodium thiosulfate solution. Calculate the percentage of iodide.

Alcoholic Potash Method—Methods for the determination of iodine in food materials have used the following means for placing the iodine in solution: digestion with sulfuric acid and hydrogen peroxide, fusion with potassium hydroxide and nitrate, and combustion tube methods. The alcoholic potash digestion method used by Almquist and Givens [16] for liquid eggs, assures in the case of liquid eggs, a more complete recovery of iodine. This method is undoubtedly applicable to other

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[15] Andrew and Mandeno, *Analyst* 60, 801 (1935).

[16] Almquist and Givens, *Ind. Eng. Chem., Anal. Ed.* 5, 254 (1933).

food materials such as meat and meat products and to milk and milk products. The treatment with alcoholic potash probably converts the organically bound iodine into iodide ion and thus stabilizes it to action of ashing.

Determination: The liquid contents of a number of eggs are placed in a flask, an equal volume of 95 per cent alcohol and 10 g. of potassium hydroxide per egg is added. The mixture is boiled under a reflux condenser, gently, for 16 to 24 hours. The boiling takes place without foaming or bumping. The product is a dark brown liquid containing very little solids. An amount of this liquid equivalent to 1 egg is placed in a 50 cc. nickel crucible or Pyrex beaker and evaporated to dryness on a hot plate. The crucible or beaker and contents are placed in a muffle furnace and ashed for about 4 hours at about 600° C.

The ash is extracted with 50 cc. of hot water, the extract is filtered off, and the residue washed with hot water, the washings being run into the filtrate. The filtrate is acidified carefully with 6 *N* sulfuric acid to the acid point of methyl red. Five more drops of acid are added. Saturated bromine water is added to the solution until it has a permanent, strong yellow color. The excess bromine is boiled off and the solution evaporated to about 15 cc. on a steam bath, cooled, and transferred to a small separatory funnel. Any crystalline matter formed during cooling and evaporating is removed and washed. The washings are added to the solution.

A crystal of potassium iodide is added and the iodine formed is extracted with five 1 cc. portions of carbon tetrachloride. The iodine in the carbon tetrachloride is determined colorimetrically by comparison with standard solutions of iodine in carbon tetrachloride. Corrections for the iodine content of the reagents used are determined by following the same procedure.

Instead of estimating the iodine colorimetrically the iodine may be determined as in the previous method using 0.1 *N* or 0.01 *N* sodium thiosulfate according to the amount of iodine liberated by the potassium iodide.

Chloride

The Volhard thiocyanate method for the determination of chloride in which the silver chloride precipitate is removed by filtration before back-titrating may be improved by eliminating the filtration. Caldwell and Moyer [17] suggest the use of nitrobenzene which inhibits the darken-

[17] Caldwell and Moyer, *Ind. Eng. Chem., Anal. Ed.* 7, 38 (1935).

ing of silver chloride in the light and improves the end point. This immiscible liquid draws the silver chloride to the interface and thus removes it from the aqueous solution, the nitrobenzene forming an insoluble layer over the precipitate.

Titrations may be made in 250 cc. glass-stoppered bottles. Twenty-five to 50 cc. of the solution containing from 0.048 to 0.26 g. of sodium chloride, free from the usual interfering ions, is acidified with 8 to 10 drops of nitric acid, and 1 cc. of nitrobenzene is added for each 0.05 g. of chloride. Standard silver nitrate is added until an excess of 1 to 4 cc. of 0.1 *N* solution is present. The bottle is then tightly stoppered and shaken vigorously until the silver chloride settles out in large spongy flakes. Usually 30–40 seconds agitation is required. A perfectly clear supernatant solution is not necessary. Fine droplets of nitrobenzene are left in suspension. However, nearly all the nitrobenzene is so closely attached to the silver chloride that there is little evidence of a separate phase.

One cc. of ferric alum indicator, prepared by adding concentrated, freshly boiled nitric acid to a saturated solution of ferric alum until the solution becomes greenish yellow, is added and the titration completed with 0.05 *N* potassium thiocyanate solution. The ferric alum acts as an effective flocculating agent and coagulates any suspended matter which is present. Standard potassium thiocyanate solution is added slowly with gentle swirling until a pink color is produced. Usually a false end point appears one drop before the true end point. It fades in about 30 seconds and may be due to the desorption of the last traces of silver nitrate from the precipitate. The next drop of thiocyanate produces a decided color change which persists 10 to 15 minutes. Titration should be made at temperatures below 25° C., as is customary in other titrations with thiocyanate. If nitric acid and subsequent boiling was used to put the material into solution, the addition of 5 cc. of a saturated solution of hydrazine sulfate just prior to the addition of the ferric alum indicator, removes any nitrous acid formed.

Diphenylcarbazide and Diphenylcarbazone Indicator for Chloride—Diphenylcarbazone was suggested as a specific indicator for the titration of chloride and bromide ions by silver ion by Chirnoaga.[18] Dubsky and Trtilek,[19] on the other hand suggested the use of both di-

[18] Chirnoaga, *Z. anal. Chem.* 101, 31 (1935).

[19] Dubsky and Trtilek, *Mikrochemie* 12, 315 (1933).

phenylcarbazide and diphenylcarbazone with mercuric nitrate as the titrating agent. This subject was reinvestigated by Roberts.[20] The method depends upon the formation, from mercuric ion and the indicator, of a deep blue-violet complex, after the chloride ions have combined to form slightly ionized mercuric chloride. Diphenylcarbazide is an acid-base indicator, changing from a light yellow in acid solution to a deep orange in alkaline solution, in the pH range of 6.6 to 7.4. It is probable that the alkaline form of the indicator forms the deep blue-violet complex with the mercuric ion.

REAGENTS: *Diphenylcarbazide*—A saturated solution of diphenylcarbazide in 95 per cent alcohol. This solution gradually turns red after standing for several days and may be used as the indicator. No apparent difference results if a fresh solution of diphenylcarbazide or diphenylcarbazone solution in alcohol is used.

Mercuric oxide: Mercuric oxide is dissolved in nitric acid (1:1) and filtered. To the filtrate is added 8 *N* sodium hydroxide until precipitation is complete. The precipitate is filtered and washed free from alkali. The yellow mercuric oxide may be dried over phosphorus pentoxide for 10 days, during which period it should be powdered.

Mercuric nitrate solutions—The required amount of mercuric oxide necessary to make 0.1 *N* and 0.025 *N* solutions of mercuric nitrate is weighed out accurately and suspended in water. The calculated equivalent amount of nitric acid is added. To the well stirred mixture nitric acid is added drop by drop until complete solution takes place. Make up to volume, and the solution should be no more than 0.01 *N* with respect to nitric acid.

Determination: With 0.1 *N* mercuric nitrate solution the following procedure should be followed: The final volume of the solution to be titrated should be about 80 to 100 cc. If the chloride solution to be titrated is acid, it should first be neutralized with 0.1 *N* sodium hydroxide solution. If the acid titer is also required, 5 drops of diphenylcarbazide is added and the solution is titrated with the standard sodium hydroxide solution to an orange color. Four cc. of 0.2 *N* nitric acid is added and the solution is then titrated with 0.1 *N* mercuric nitrate solution. About 5 drops before the end point, a pink-violet color begins to develop. At the end point, one drop changes the color from a light violet to a deep blue violet.

If the chloride solution is dilute and requires 0.025 *N* mercuric nitrate

[20] Roberts, *Ind. Eng. Chem., Anal. Ed.* 8, 365 (1937).

solution additional precautions need be observed. The final volume should be 65 ± 10 cc. If the chloride solution to be titrated is acid, 2 drops of 0.2 per cent bromophenol blue is added, and the solution is titrated with standard sodium hydroxide solution to the full blue color. Four cc. of 0.2 *N* nitric acid is added, then 5 drops of the diphenylcarbazide indicator, and the solution is titrated with 0.025 *N* mercuric nitrate solution to a definite pink color, which can be reproduced to ±0.02 cc., with the aid of a daylight lamp. The yellow color imparted by the bromophenol blue in no way interferes with the mercuric nitrate end point, and to make conditions uniform for all titrations 2 drops of bromophenol blue should be added whenever 0.025 *N* mercuric nitrate solution is used.

A blank correction should be determined with the 2 drops of bromophenol blue, 4 cc. of 0.2 *N* nitric acid and with the nitric acid equivalent to the amount of acid in the mercuric nitrate solution used in the titration.

Nitrates and Nitrites

The following method is based on the nitration of phenoldisulfonic acid by any nitrate present with the formation of a colored nitrophenoldisulfonic acid compound. Any nitrite present is oxidized to nitrate and reacts the same way.

Phenoldisulfonic Acid Method—The A. O. A. C. gives the following details: *Reagents*—a) Phenoldisulfonic acid solution.—Heat 6 g. of phenol with 37 cc. of sulfuric acid on a steam bath, cool, and add 3 cc. of water.

b) Standard comparison solution.—Dissolve 1 g. of pure, dry sodium nitrate in water and dilute to 1 liter. Evaporate 10 cc. of this solution to dryness on a steam bath, add 2 cc. of the reagent (a), mix quickly and thoroughly by means of a glass rod, heat for about a minute on a steam bath, and dilute to 100 cc. One cc. of the dilute solution = 0.1 mg. of sodium nitrate. Prepare a series of standard comparison tubes by introducing quantities ranging from 1 to 20 cc. of the diluted solution (0.1–20 mg. of sodium nitrate) into 50 cc. Nessler tubes, adding 5 cc. of ammonium hydroxide to each and diluting to 50 cc. The standard tubes thus prepared are permanent for several weeks if kept tightly stoppered.

Determination: Weigh 1 g. of the sample into a 100 cc. flask, add 20–30 cc. of water, and heat on a steam bath for 15 minutes, shaking occasionally. Add 3 cc. of a saturated nitrate-free silver sulfate solution for each per cent of sodium chloride present, then 10 cc. of basic lead

acetate solution and 5 cc. of alumina cream, shaking after each addition. Make up to the mark with water, shake, and filter through a folded filter, returning the filtrate to the filter until it runs through clear. Evaporate 25 cc. of the filtrate to dryness, add 1 cc. of the phenoldisulfonic acid solution, mix quickly and thoroughly by means of a glass rod, add 1 cc. of water and 3–4 drops of sulfuric acid, and heat on a steam bath for 2–3 minutes, being careful not to char the material. Then add about 25 cc. of water and an excess of ammonium hydroxide, transfer to a 100 cc. volumetric flask, add 1–2 cc. of alumina cream if not perfectly clear, dilute to volume with water, and filter. Fill a 50 cc. Nessler tube to the mark with the filtrate and determine the quantity of sodium nitrate present in the sample by comparison with the standard comparison tubes. If the solution is too dark for comparison with the standards, dilute with water, and correct the result accordingly.

Nitrates in Flesh Foods

Van Voorst [21] suggests the following procedure. The sample is broken up well and 10 g. are heated, once with 100 cc. and then with 4–50 cc. portions of water, the combined extracts are diluted to 500 cc. They are filtered cold and the chlorides are removed from a 25 cc. aliquot by the addition of 10 cc. of 0.5 per cent silver sulfate solution. Ten cc. of the resulting filtrate is evaporated to dryness. The residue is dissolved in 5 cc. of water and the solution is allowed to stand for 10 minutes with 1 cc. of a solution of 3 g. of phenol in 32 g. of 96 per cent sulfuric acid. The mixture is made ammoniacal and the yellow color produced is matched in a volume of 50 cc. against that produced from a known volume of a solution of 60 mg. potassium nitrate in 250 cc. of water, treated the same way. The color due to nitrite is negligible. Good results may be obtained in sausage containing from 0.1 to 1 per cent potassium nitrate.

Nitrite

The nitrite ion present is used to diazotize some added sulfanilic acid which then is coupled with α-naphthylamine hydrochloride. *Reagents:* (a) Sulfanilic acid solution.—Dissolve 1 g. of sulfanilic acid in hot water, cool, and dilute to 100 cc.

(b) α-naphthylamine hydrochloride solution.—Boil 0.5 g. of the salt with 100 cc. of water, kept at constant volume, for 10 minutes.

[21] Van Voorst, *Chem. Weekblad* 30, 101 (1933).

(c) Standard nitrite solution.—Dissolve 1.1 g. of silver nitrite in nitrite-free water, precipitate the silver with sodium chloride solution, dilute to 1 liter, mix, allow to settle. Dilute 100 cc. of the supernatant liquid to 1 liter and then 10 cc. of this solution to 1 liter, using in each case nitrite-free water. One cc. of the final dilution = 0.0001 mg. of nitrogen as nitrite.

Determination: Weigh 5 g. of the sample into a 50 cc. beaker. Add approximately 40 cc. of nitrite-free water heated to a temperature of 80° C. Mix thoroughly by stirring with a glass rod, taking care to break up all lumps, and transfer to a 500 cc. graduated flask. Wash out the beaker and rod thoroughly with successive portions of the hot water, adding all washings to the flask. Add sufficient hot water to bring the contents of the flask to a volume of approximately 300 cc., transfer the flask to the steam bath, and let stand for 2 hours, shaking occasionally. Add 5 cc. of saturated mercuric chloride solution and mix. Cool to room temperature, make up to the mark with nitrite-free water and mix again. Filter and determine nitrogen as nitrite in a suitable aliquot.

Place 100 cc. of the suitably diluted aliquot in a 100 cc. Nessler tube and treat with 1 or 2 drops of hydrochloric acid. Add 1 cc. of the sulfanilic acid solution, 1 cc. of the α-naphthylamine hydrochloride solution, and mix thoroughly. Set aside for 30 minutes with other Nessler tubes containing known quantities of the standard nitrite solution made up to 100 cc. with the nitrite-free water and treated with hydrochloric acid, sulfanilic acid, and α-naphthylamine hydrochloride solutions in the same manner as the sample. Determine the quantity of the nitrite by comparison with the depth of pink color in the known and unknown solutions.

Germuth [22] recommends that because of the instability of the α-naphthylamine complex, that dimethyl-α-naphthylamine $C_{10}H_7N:(CH_3)_2$ be substituted for the α-naphthylamine. A solution of 5.25 g. of dimethyl-α-naphthylamine dissolved in 1 liter of 4 *N* acetic acid in 95 per cent methanol insures the most satisfactory results in the colorimetric estimation of nitrites. Such a solution is stable and is used in exactly the same manner as the α-naphthylamine as directed above.

Ammonia

Uncombined ammonia may be estimated by aeration in preference to distillation, for boiling even in dilute alkali, at times, will cause some

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[22] Germuth, *Ind. Eng. Chem., Anal. Ed.* 1, 28 (1929).

decomposition with the formation of more ammonia. This procedure is generally applied to flesh products. The ammonia is liberated by sodium or potassium carbonate solution and is then aspirated into a measured quantity of standard acid. The ammonia is then estimated by titrating the excess acid with standard alkali solution or by nesslerizing.

In the wash bottle on the right in Fig. 56 is placed 125 cc. of sulfuric acid (1:9) which is used to wash the incoming air. In the adjoining test tube, place a weighed quantity of sample of from 2 to 4 g. and 20 cc. of ammonia-free water. In the next test tube introduce a measured

FIG. 56. Ammonia Aeration Apparatus

quantity of 0.02 to 0.05 N sulfuric or hydrochloric acid, according to the amount of ammonia evolved. The end wash bottle is used as a safety bottle.

Add to the sample 1 cc. of saturated potassium oxalate solution and a few drops of kerosene to minimize frothing. Then add potassium or sodium carbonate solution until the mixture is just alkaline. Replace the tubes in position immediately and pass air through the system by means of an aspirator. Titrate the standard acid at hourly intervals

until ammonia ceases to be evolved using methyl red as the indicator or else nesslerize as described in the Farinacci method in Chapter I.

Sodium

With the introduction of the zinc uranyl acetate reagent for sodium by Barber and Kolthoff [23] a certain specificity was obtained in analyses for sodium. The *reagent* is made from two solutions. Solution (1) is composed of 100 g. of uranyl acetate, $UO_2(OAc)_2.2H_2O$, 60 g. of acetic acid and sufficient water to make 650 g. Solution (2) is composed of 300 g. of zinc acetate, $Zn(OAc)_2.3H_2O$, 30 g. of 30 per cent acetic acid and sufficient water to make 650 g. After the salts in solutions (1) and (2) are dissolved by warming on a steam bath to 70° C., the solutions are mixed, a few mg. of sodium chloride are added to saturate the solution with sodium and the reagent is allowed to stand for 24 hours. The solution is filtered immediately before use.

In reducing the volume of sodium solution to 1 or 2 cc., which is necessary before the addition of the zinc uranyl acetate reagent, salts may crystallize out if the reagents used to remove phosphates, arsenates and other radicals yield salts that are not very soluble. Overman and Garrett [24] circumvent this difficulty by the use of zinc carbonate.

Determination: Transfer a 10 to 15 cc. aliquot of solution containing from 2 to 8 mg. of sodium in a 50 cc. beaker. Add an excess of powdered zinc carbonate, cover the beaker, and let stand at room temperature overnight. If too much hydrochloric acid is present, the violent effervescence may cause loss of material or zinc chloride may possibly crystallize out in the evaporation. Hence if hydrochloric acid is present in large amounts, evaporate to dryness, add 10 cc. of water and just sufficient hydrochloric acid to put the salts into solution. Then add the zinc carbonate. Filter through quantitative paper in a small funnel and wash thoroughly with cold water 5 or 6 times with small portions of water. Catch the filtrate and washings in a small beaker and evaporate to 1 or 2 cc. Add 100 cc. of the zinc uranyl acetate reagent and stir vigorously for an hour or allow to stand overnight. Filter through a weighed Gooch crucible, wash well with 95 per cent alcohol saturated with the sodium-zinc uranyl acetate salt and then with ether saturated with the same salt. Dry in air and weigh. The salt has the formula: $(UO_2)_3ZnNa(OAc)_9.6H_2O$.

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[23] Barber and Kolthoff, *J. Am. Chem. Soc.* 50, 1625 (1928).

[24] Overman and Garrett, *Ind. Eng. Chem., Anal. Ed.* 9, 72 (1937).

Caley and Foulk Magnesium Uranyl Acetate Method —Caley and Foulk [25] suggested the use of the complex reagent magnesium uranyl acetate instead of zinc uranyl acetate. This reagent yields a precipitate of $(UO_2)_3MgNa(OAc)_9.6\frac{1}{2}H_2O$.

The reagent is also made from two solutions. Solution 1 consists of 85 g. uranyl acetate, $UO_2(OAc)_2.2H_2O$, 60 g. of glacial acetic acid and sufficient water to make a liter. Solution 2 consists of 500 g. of magnesium acetate, $Mg(OAc)_2.4H_2O$, 60 g. of glacial acetic acid and sufficient water to make a liter. The two solutions are heated separately at approximately 70° C. on a water or steam bath until the salts are dissolved. The solutions are then mixed at that temperature and the mixture is allowed to cool to 20° C. Add a few mg. of sodium chloride and then allow the mixture to stand at 20° C. overnight. Filter and catch the reagent in a dry bottle.

Determination: The sodium solution may be prepared as directed above or the following procedure may be used.

Add to the sample of which 1–10 g. is used, according to the sodium content, sufficient sulfuric acid (1:10) to make the sample acid and dry. Ash in a muffle at low red heat. Dissolve the residue in about 2–5 cc. of hydrochloric acid by warming on a water bath, add about 40 cc. of water and heat to boiling. Add sufficient calcium chloride to precipitate all the phosphates and make the precipitation complete by the addition of ammonium hydroxide until the mixture is slightly alkaline. Filter through a small filter. Wash thoroughly and catch the filtrate and washings in a 150 cc. beaker. Evaporate the filtrate to 1 or 2 cc. or if salts crystallize out, only to 5 cc. Cool and add 100 cc. of the magnesium uranyl acetate reagent. Stir vigorously and allow to stand overnight. Filter through a weighed Gooch crucible and wash the precipitate with 95 per cent alcohol and ether saturated with the magnesium sodium uranyl acetate salt. Dry at 105–110° C. for 30 minutes, cool and weigh. Weight of sodium-magnesium uranyl acetate × 0.0153 = sodium. The presence of small quantities of ammonium and potassium do not interfere but lithium does.

The sodium may be estimated colorimetrically by dissolving the precipitate in hot water. A yellow solution is formed which may be compared in a colorimeter against known amounts of sodium prepared the same way.

[25] Caley and Foulk, *J. Am. Chem. Soc.* **51**, 1664 (1929).

Potassium

It is at times necessary for the food analyst to detect and estimate potassium in foodstuffs. The flame test, precipitation as the chlorplatinate and cobaltinitrite are the usual tests. Clark and Willits [26] suggest that naphthol yellow S may be used for the detection of potassium for naphthol yellow S; that is, the disodium salt of 2:4 dinitro-1-naphthol-7-sulfonic acid forms a difficultly soluble lake with potassium.

To 10 cc. of the aqueous solution to be tested, containing only the soluble group, add 3 cc. of 2 per cent solution of naphthol yellow S and set aside at room temperature. The appearance of a precipitate within 65 minutes or less indicates the presence of at least 0.79 mg. of potassium per liter. Three cc. of a 5 per cent solution of the dye should produce a precipitate with 0.39 mg. of potassium per liter in 20 minutes or less.

In order to test for potassium and sodium separately on the same solution Adams, Hall and Bailey [27] suggest the use of zinc cobaltinitrite as a reagent for potassium. The reagent of zinc cobaltinitrite may be prepared by passing the oxides of nitrogen obtained by the action of nitric acid on copper foil through a solution of cobalt acetate and zinc acetate for from 45 to 60 minutes. The resulting brown solution is kept in brown bottles tightly stoppered and is decanted from any precipitate formed.

To the solution containing no barium, arsenate, phosphate or ammonium, add an equal volume of zinc cobaltinitrite and allow to stand for 15 minutes. A yellow precipitate of potassium cobaltinitrite indicates potassium. The filtrate may be used for the detection of sodium by the uranyl acetate method.

Wilcox [28] uses an aqueous solution of trisodium cobaltinitrite in the presence of nitric acid as the precipitating agent for potassium. He points out the following advantages for the method: The precipitate is crystalline and heavy, comparing favorably with barium sulfate in ease of filtering and washing. The composition appears to be constant and practically independent of the sodium ion concentration. The determination can be completed either gravimetrically by drying and weighing the precipitate or volumetrically by titration with potassium permanganate. The volumetric method depends upon the oxidation of the nitrite of the precipitate with permanganate in acid solution. The co-

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[26] Clark and Willits, *Ind. Eng. Chem., Anal. Ed.* **8**, 209 (1936).

[27] Adams, Hall and Bailey, *Ind. Eng. Chem., Anal. Ed.* **7**, 310 (1935).

[28] Wilcox, *Ind. Eng. Chem., Anal. Ed.* **9**, 136 (1937).

balt is present as Co^{+++} and under these conditions is a strong oxidizing agent equal to potassium permanganate and that it therefore oxidizes an equivalent amount of nitrite. The reaction:

$$5K_2NaCo(NO_2)_6 + 11KMnO_4 + 14H_2SO_4 \rightarrow 5CoSO_4 + 9MnSO_4 + 2Mn(NO_3)_2 + 5NaNO_3 + 21KNO_3 + 14H_2O$$

Here 30 NO_2 or 60 reducing equivalents are balanced by 11 $KMnO_4$ or 55 oxidizing equivalents plus 5 oxidizing equivalents from the cobaltic-cobaltous couple. Therefore 11 $KMnO_4$ or 55 equivalents are required for 10 K or

$$\frac{10K}{55} = \frac{390.96}{55} = 7.1084 \text{ g. of } K \text{ per equivalent of } KMnO_4.$$

Gravimetric Determination: Prepare an aqueous solution containing 1 g. of the trisodium cobaltinitrite in each 5 cc., allowing 5 cc. for each determination. Filter before use. Prepare freshly if necessary.

The aliquot for analysis should contain between 2 and 15 mg. of potassium in a neutral aqueous solution of 10 cc. volume. Add 1 cc. of 1 *N* nitric acid and 5 cc. of the sodium cobaltinitrite solution, mix, and allow to stand for 2 hours. Filter in a porous-bottomed porcelain crucible, the tare weight of which is known, using 0.01 *N* nitric acid in a wash bottle to make the transfer. Wash 10 times with 2 cc. portions of the dilute nitric acid and 5 times with 2 cc. portions of 95 per cent alcohol. Evacuate until dry. Wipe the outside with a cloth, and dry for 1 hour at 110° C. Cool, in a desiccator and weigh.

The composition of the precipitate can be represented by the formula $K_2NaCo(NO_2)_6.H_2O$. $K = 17.216\%$.

Volumetric Determination: Follow the gravimetric procedure through the precipitation and washing with nitric acid. Omit washing with alcohol. Wash the precipitate into a 250 cc. beaker, place the crucible in the beaker, and make to about 100 cc. with water. Add 20 cc. of 0.5 *N* sodium hydroxide solution and boil for 3 minutes. Withdraw into another beaker a slight excess of standard 0.05 *N* potassium permanganate, make to 50 cc. with water, and add 5 cc. sulfuric acid. Pour the hot potassium cobaltinitrite solution into the cold potassium permanganate solution, transfer the crucible and wash the beaker with a small amount of water. Add an excess of standard 0.05 *N* sodium oxalate solution, heat to boiling and complete the titration with potassium permanganate.

Cc. of $KMnO_4$ × normality of $KMnO_4 \times 7.1084$ = mg. of K in sample titrated.

There are many colorimetric methods for the determination of potassium based on the estimation of the nitrite content in the potassium cobaltinitrite precipitate.

Potassium forms a slightly soluble, yellow to dark red salt with dipicrylamine. The potassium is precipitated in solutions neutral to methyl red by the addition of 0.5 normal solution of the magnesium salt of dipicrylamine.[29]

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[29] Winkel and Mass, *Z. angew. Chem.* **49**, 827 (1936).

APPENDIX TABLES

TABLE 1. MUNSON AND WALKER SUGAR TABLE[1,2] (in milligrams)

Cuprous oxide (Cu_2O)	Copper (Cu)	Dextrose (*d*-glucose)	Invert sugar	Invert sugar and sucrose: 0.4 gram total sugar	Invert sugar and sucrose: 2 grams total sugar	Lactose: $C_{12}H_{22}O_{11}+H_2O$	Lactose and sucrose: 1 lactose, 4 sucrose	Lactose and sucrose: 1 lactose, 12 sucrose	Maltose: $C_{12}H_{22}O_{11}+H_2O$	Cuprous oxide (Cu_2O)
10	8.9	4.0	4.5	1.6	......	6.3	6.1	......	6.2	10
12	10.7	4.9	5.4	2.5	......	7.5	7.3	......	7.9	12
14	12.4	5.7	6.3	3.4	......	8.8	8.5	......	9.5	14
16	14.2	6.6	7.2	4.3	......	10.0	9.7	......	11.2	16
18	16.0	7.5	8.1	5.2	......	11.3	10.9	......	12.9	18
20	17.8	8.3	8.9	6.1	......	12.5	12.1	......	14.6	20
22	19.5	9.2	9.8	7.0	......	13.8	13.3	......	16.2	22
24	21.3	10.0	10.7	7.9	......	15.0	14.5	......	17.9	24
26	23.1	10.9	11.6	8.8	......	16.3	15.8	......	19.6	26
28	24.9	11.8	12.5	9.7	......	17.6	17.0	......	21.2	28
30	26.6	12.6	13.4	10.7	4.3	18.8	18.2	......	22.9	30
32	28.4	13.5	14.3	11.6	5.2	20.1	19.4	......	24.6	32
34	30.2	14.3	15.2	12.5	6.1	21.4	20.7	......	26.2	34
36	32.0	15.2	16.1	13.4	7.0	22.8	22.0	......	27.9	36
38	33.8	16.1	16.9	14.3	7.9	24.2	23.3	......	29.6	38
40	35.5	16.9	17.8	15.2	8.8	25.5	24.7	......	31.3	40
42	37.3	17.8	18.7	16.1	9.7	26.9	26.0	......	32.9	42
44	39.1	18.7	19.6	17.0	10.7	28.3	27.3	......	34.6	44
46	40.9	19.6	20.5	17.9	11.6	29.6	28.6	......	36.3	46
48	42.6	20.4	21.4	18.8	12.5	31.0	30.0	......	37.9	48
50	44.4	21.3	22.3	19.7	13.4	32.3	31.3	......	39.6	50
52	46.2	22.2	23.2	20.7	14.3	33.7	32.6	......	41.3	52
54	48.0	23.0	24.1	21.6	15.2	35.1	34.0	......	42.9	54
56	49.7	23.9	25.0	22.5	16.2	36.4	35.3	......	44.6	56
58	51.5	24.8	25.9	23.4	17.1	37.8	36.6	......	46.3	58
60	53.3	25.6	26.8	24.3	18.0	39.2	37.9	......	48.0	60
62	55.1	26.5	27.7	25.2	18.9	40.5	39.3	......	49.6	62
64	56.8	27.4	28.6	26.2	19.8	41.9	40.6	......	51.3	64
66	58.6	28.3	29.5	27.1	20.8	43.3	41.9	......	53.0	66
68	60.4	29.2	30.4	28.0	21.7	44.7	43.3	40.7	54.6	68
70	62.2	30.0	31.3	28.9	22.6	46.0	44.6	41.9	56.3	70
72	64.0	30.9	32.3	29.8	23.5	47.4	45.9	43.1	58.0	72
74	65.7	31.8	33.2	30.8	24.5	48.8	47.3	44.2	59.6	74
76	67.5	32.7	34.1	31.7	25.4	50.1	48.6	45.4	61.3	76
78	69.3	33.6	35.0	32.6	26.3	51.5	49.9	46.6	63.0	78
80	71.1	34.4	35.9	33.5	27.3	52.9	51.3	47.8	64.6	80
82	72.8	35.3	36.8	34.5	28.2	54.2	52.6	49.0	66.3	82
84	74.6	36.2	37.7	35.4	29.1	55.6	53.9	50.1	68.0	84
86	76.4	37.1	38.6	36.3	30.0	57.0	55.3	51.3	69.7	86
88	78.2	38.0	39.5	37.2	31.0	58.4	56.6	52.5	71.3	88

[1] U. S. Bureau Standards, Circ. 44 (1918).

[2] Given, "Methods of Sugar Analysis and Allied Determinations," Blakiston (1912).

TABLE 1. MUNSON AND WALKER SUGAR TABLE (in milligrams)
—*Continued*

CUPROUS OXIDE (Cu_2O)	COPPER (Cu)	DEXTROSE (*d*-GLUCOSE)	INVERT SUGAR	INVERT SUGAR AND SUCROSE		LACTOSE	LACTOSE AND SUCROSE		MALTOSE	CUPROUS OXIDE (Cu_2O)
				0.4 gram total sugar	2 grams total sugar	$C_{12}H_{22}O_{11}+H_2O$	1 lactose, 4 sucrose	1 lactose, 12 sucrose	$C_{12}H_{22}O_{11}+H_2O$	
90	79.9	38.9	40.4	38.2	31.9	59.7	57.9	53.7	73.0	90
92	81.7	39.8	41.4	39.1	32.8	61.1	59.3	54.9	74.7	92
94	83.5	40.6	42.3	40.0	33.8	62.5	60.6	56.0	76.3	94
96	85.3	41.5	43.2	41.0	34.7	63.8	61.9	57.2	78.0	96
98	87.1	42.4	44.1	41.9	35.6	65.2	63.3	58.4	79.7	98
100	88.8	43.3	45.0	42.8	36.6	66.6	64.6	59.6	81.3	100
102	90.6	44.2	46.0	43.8	37.5	68.0	66.0	60.8	83.0	102
104	92.4	45.1	46.9	44.7	38.5	69.3	67.3	62.0	84.7	104
106	94.2	46.0	47.8	45.6	39.4	70.7	68.6	63.2	86.3	106
108	95.9	46.9	48.7	46.6	40.3	72.1	70.0	64.4	88.0	108
110	97.7	47.8	49.6	47.5	41.3	73.5	71.3	65.6	89.7	110
112	99.5	48.7	50.6	48.4	42.2	74.8	72.6	66.7	91.3	112
114	101.3	49.6	51.5	49.4	43.2	76.2	74.0	67.9	93.0	114
116	103.0	50.5	52.4	50.3	44.1	77.6	75.3	69.1	94.7	116
118	104.8	51.4	53.3	51.2	45.0	79.0	76.7	70.3	96.4	118
120	106.6	52.3	54.3	52.2	46.0	80.3	78.0	71.5	98.0	120
122	108.4	53.2	55.2	53.1	46.9	81.7	79.3	72.7	99.7	122
124	110.1	54.1	56.1	54.1	47.9	83.1	80.7	73.9	101.4	124
126	111.9	55.0	57.0	55.0	48.8	84.5	82.0	75.1	103.0	126
128	113.7	55.9	58.0	55.9	49.8	85.8	83.4	76.3	104.7	128
130	115.5	56.8	58.9	56.9	50.7	87.2	84.7	77.5	106.4	130
132	117.3	57.7	59.8	57.8	51.7	88.6	86.0	78.7	108.0	132
134	119.0	58.6	60.8	58.8	52.6	90.0	87.4	79.7	109.7	134
136	120.8	59.5	61.7	59.7	53.6	91.3	88.7	81.1	111.4	136
138	122.6	60.4	62.6	60.7	54.5	92.7	90.1	82.3	113.0	138
140	124.4	61.3	63.6	61.6	55.5	94.1	91.4	83.5	114.7	140
142	126.1	62.2	64.5	62.6	56.4	95.5	92.8	84.7	116.4	142
144	127.9	63.1	65.4	63.5	57.4	96.8	94.1	85.9	118.0	144
146	129.7	64.0	66.4	64.5	58.3	98.2	95.4	87.1	119.7	146
148	131.5	65.0	67.3	65.4	59.3	99.6	96.8	88.3	121.4	148
150	133.2	65.9	68.3	66.4	60.2	101.0	98.1	89.5	123.0	150
152	135.0	66.8	69.2	67.3	61.2	102.3	99.5	90.8	124.7	152
154	136.8	67.7	70.1	68.3	62.1	103.7	100.8	92.0	126.4	154
156	138.6	68.6	71.1	69.2	63.1	105.1	102.2	93.2	128.0	156
158	140.3	69.5	72.0	70.2	64.1	106.5	103.5	94.4	129.7	158
160	142.1	70.4	73.0	71.2	65.0	107.9	104.8	95.6	131.4	160
162	143.9	71.4	73.9	72.1	66.0	109.2	106.2	96.8	133.0	162
164	145.7	72.3	74.9	73.1	66.9	110.6	107.5	98.0	134.7	164
166	147.5	73.2	75.8	74.0	67.9	112.0	108.9	99.2	136.4	166
168	149.2	74.1	76.8	75.0	68.9	113.4	110.2	100.4	138.0	168

TABLE 1. MUNSON AND WALKER SUGAR TABLE (in milligrams)
—*Continued*

Cuprous oxide (Cu_2O)	Copper (Cu)	Dextrose (*d*-glucose)	Invert sugar	Invert sugar and sucrose: 0.4 gram total sugar	Invert sugar and sucrose: 2 grams total sugar	Lactose: $C_{12}H_{22}O_{11}+H_2O$	Lactose and sucrose: 1 lactose, 4 sucrose	Lactose and sucrose: 1 lactose, 12 sucrose	Maltose: $C_{12}H_{22}O_{11}+H_2O$	Cuprous oxide (Cu_2O)
170	151.0	75.1	77.7	76.0	69.8	114.8	111.6	101.6	139.7	170
172	152.8	76.0	78.7	76.9	70.8	116.1	112.9	102.8	141.4	172
174	154.6	76.9	79.6	77.9	71.7	117.5	114.3	104.1	143.0	174
176	156.3	77.8	80.6	78.8	72.7	118.9	115.6	105.3	144.7	176
178	158.1	78.8	81.5	79.8	73.7	120.3	117.0	106.5	146.4	178
180	159.9	79.7	82.5	80.8	74.6	121.6	118.3	107.7	148.0	180
182	161.7	80.6	83.4	81.7	75.6	123.1	119.7	108.9	149.7	182
184	163.4	81.5	84.4	82.7	76.6	124.3	121.0	110.1	151.4	184
186	165.2	82.5	85.3	83.7	77.6	125.8	122.4	111.3	153.0	186
188	167.0	83.4	86.3	84.6	78.5	127.2	123.7	112.5	154.7	188
190	168.8	84.3	87.2	85.6	79.5	128.5	125.1	113.8	156.4	190
192	170.5	85.3	88.2	86.6	80.5	129.9	126.4	115.0	158.0	192
194	172.3	86.2	89.2	87.6	81.4	131.3	127.8	116.2	159.7	194
196	174.1	87.1	90.1	88.5	82.4	132.7	129.2	117.4	161.4	196
198	175.9	88.1	91.1	89.5	83.4	134.1	130.5	118.6	163.0	198
200	177.7	89.0	92.0	90.5	84.4	135.4	131.9	119.8	164.7	200
202	179.4	89.9	93.0	91.4	85.3	136.8	133.2	121.0	166.4	202
204	181.2	90.9	94.0	92.4	86.3	138.2	134.6	122.3	168.0	204
206	183.0	91.8	94.9	93.4	87.3	139.6	135.9	123.5	169.7	206
208	184.8	92.8	95.9	94.4	88.3	141.0	137.3	124.7	171.4	208
210	186.5	93.7	96.9	95.4	89.2	142.3	138.6	126.0	173.0	210
212	188.3	94.6	97.8	96.3	90.2	143.7	140.0	127.2	174.7	212
214	190.1	95.6	98.8	97.3	91.2	145.1	141.4	128.4	176.4	214
216	191.9	96.5	99.8	98.3	92.2	146.5	142.7	129.6	178.0	216
218	193.6	97.5	100.8	99.3	93.2	147.9	144.1	130.9	179.7	218
220	195.4	98.4	101.7	100.3	94.2	149.3	145.4	132.1	181.4	220
222	197.2	99.4	102.7	101.2	95.1	150.7	146.8	133.3	183.0	222
224	199.0	100.3	103.7	102.2	96.1	152.0	148.1	134.5	184.7	224
226	200.7	101.3	104.6	103.2	97.1	153.4	149.5	135.8	186.4	226
228	202.5	102.2	105.6	104.2	98.1	154.8	150.8	137.0	188.0	228
230	204.3	103.2	106.6	105.2	99.1	156.2	152.2	138.2	189.7	230
232	206.1	104.1	107.6	106.2	100.1	157.6	153.6	139.4	191.3	232
234	207.9	105.1	108.6	107.2	101.1	159.0	154.9	140.7	193.0	234
236	209.6	106.0	109.5	108.2	102.1	160.3	156.3	141.9	194.7	236
238	211.4	107.0	110.5	109.2	103.1	161.7	157.6	143.2	196.3	238
240	213.2	108.0	111.5	110.1	104.0	163.1	159.0	144.4	198.0	240
242	215.0	108.9	112.5	111.1	105.0	164.5	160.3	145.6	199.7	242
244	216.7	109.9	113.5	112.1	106.0	165.9	161.7	146.9	201.3	244
246	218.5	110.8	114.5	113.1	107.0	167.3	163.1	148.1	203.0	246
248	220.3	111.8	115.4	114.1	108.0	168.7	164.4	149.3	204.7	248

TABLE 1. MUNSON AND WALKER SUGAR TABLE (in milligrams)
—*Continued*

Cuprous oxide (Cu_2O)	Copper (Cu)	Dextrose (d-glucose)	Invert sugar	Invert sugar and sucrose: 0.1 gram total sugar	Invert sugar and sucrose: 2 grams total sugar	Lactose: $C_{12}H_{22}O_{11}+H_2O$	Lactose and sucrose: 1 lactose, 4 sucrose	Lactose and sucrose: 1 lactose, 12 sucrose	Maltose: $C_{12}H_{22}O_{11}+H_2O$	Cuprous oxide (Cu_2O)
250	222.1	112.8	116.4	115.1	109.0	170.1	165.8	150.6	206.3	250
252	223.8	113.7	117.4	116.1	110.0	171.5	167.2	151.8	208.0	252
254	225.6	114.7	118.4	117.1	111.0	172.8	168.5	153.1	209.7	254
256	227.4	115.7	119.4	118.1	112.0	174.2	169.9	154.3	211.3	256
258	229.2	116.6	120.4	119.1	113.0	175.6	171.3	155.5	213.0	258
260	231.0	117.6	121.4	120.1	114.0	177.0	172.6	156.8	214.7	260
262	232.7	118.6	122.4	121.1	115.0	178.4	174.0	158.0	216.3	262
264	234.5	119.5	123.4	122.1	116.0	179.8	175.3	159.3	218.0	264
266	236.3	120.5	124.4	123.1	117.0	181.2	176.7	160.5	219.7	266
268	238.1	121.5	125.4	124.1	118.0	182.6	178.1	161.8	221.3	268
270	239.8	122.5	126.4	125.1	119.0	184.0	179.4	163.0	223.0	270
272	241.6	123.4	127.4	126.2	120.0	185.3	180.8	164.3	224.6	272
274	243.4	124.4	128.4	127.2	121.1	186.7	182.2	165.5	226.3	274
276	245.2	125.4	129.4	128.2	122.1	188.1	183.5	166.8	228.0	276
278	246.9	126.4	130.4	129.2	123.1	189.5	184.9	168.0	229.6	278
280	248.7	127.3	131.4	130.2	124.1	190.9	186.3	169.3	231.3	280
282	250.5	128.3	132.4	131.2	125.1	192.3	187.6	170.5	233.0	282
284	252.3	129.3	133.4	132.2	126.1	193.7	189.0	171.8	234.6	284
286	254.0	130.3	134.4	133.2	127.1	195.1	190.4	173.0	236.3	286
288	255.8	131.3	135.4	134.3	128.1	196.5	191.7	174.3	238.0	288
290	257.6	132.3	136.4	135.3	129.2	197.8	193.1	175.5	239.6	290
292	259.4	133.2	137.4	136.3	130.2	199.2	194.4	176.8	241.3	292
294	261.2	134.2	138.4	137.3	131.2	200.6	195.8	178.1	242.9	294
296	262.9	135.2	139.4	138.3	132.2	202.0	197.2	179.3	244.6	296
298	264.7	136.2	140.5	139.4	133.2	203.4	198.6	180.6	246.3	298
300	266.5	137.2	141.5	140.4	134.2	204.8	199.9	181.8	247.9	300
302	268.3	138.2	142.5	141.4	135.3	206.2	201.3	183.1	249.6	302
304	270.0	139.2	143.5	142.4	136.3	207.6	202.7	184.4	251.3	304
306	271.8	140.2	144.5	143.4	137.3	209.0	204.0	185.6	252.9	306
308	273.6	141.2	145.5	144.5	138.3	210.4	205.4	186.9	254.6	308
310	275.4	142.2	146.6	145.5	139.4	211.8	206.8	188.1	256.3	310
312	277.1	143.2	147.6	146.5	140.4	213.2	208.1	189.4	257.9	312
314	278.9	144.2	148.6	147.6	141.4	214.6	209.5	190.7	259.6	314
316	280.7	145.2	149.6	148.6	142.4	216.0	210.9	191.9	261.2	316
318	282.5	146.2	150.7	149.6	143.5	217.3	212.2	193.2	262.9	318
320	284.2	147.2	151.7	150.7	144.5	218.7	213.6	194.4	264.6	320
322	286.0	148.2	152.7	151.7	145.5	220.1	215.5	195.7	266.2	322
324	287.8	149.2	153.7	152.7	146.6	221.5	216.4	197.0	267.9	324
326	289.6	150.2	154.8	153.8	147.6	222.9	217.7	198.2	269.6	326
328	291.4	151.2	155.8	154.8	148.6	224.3	219.1	199.5	271.2	328

TABLE 1. MUNSON AND WALKER SUGAR TABLE (in milligrams)
—*Continued*

Cuprous oxide (Cu_2O)	Copper (Cu)	Dextrose (*d*-glucose)	Invert sugar	Invert sugar and sucrose: 0.4 gram total sugar	Invert sugar and sucrose: 2 grams total sugar	Lactose: $C_{12}H_{22}O_{11}+H_2O$	Lactose and sucrose: 1 lactose, 4 sucrose	Lactose and sucrose: 1 lactose, 12 sucrose	Maltose: $C_{12}H_{22}O_{11}+H_2O$	Cuprous oxide (Cu_2O)
330	293.1	152.2	156.8	155.8	149.7	225.7	220.5	200.8	272.9	330
332	294.9	153.2	157.9	156.9	150.7	227.1	221.8	202.0	274.6	332
334	296.7	154.2	158.9	157.9	151.7	228.5	223.2	203.3	276.2	334
336	298.5	155.2	159.9	159.0	152.8	229.9	224.6	204.6	277.9	336
338	300.2	156.3	161.0	160.0	153.8	231.3	226.0	205.9	279.5	338
340	302.0	157.3	162.0	161.0	154.8	232.7	227.4	207.1	281.2	340
342	303.8	158.3	163.1	162.1	155.9	234.1	228.7	208.4	282.9	342
344	305.6	159.3	164.1	163.1	156.9	235.5	230.1	209.7	284.5	344
346	307.3	160.3	165.1	164.2	158.0	236.9	231.5	211.0	286.2	346
348	309.1	161.4	166.2	165.2	159.0	238.3	232.9	212.2	287.9	348
350	310.9	162.4	167.2	166.3	160.1	239.7	234.3	213.5	289.5	350
352	312.7	163.4	168.3	167.3	161.1	241.1	235.6	214.8	291.2	352
354	314.4	164.4	169.3	168.4	162.2	242.5	237.0	216.1	292.8	354
356	316.2	165.4	170.4	169.4	163.2	243.9	238.4	217.3	294.5	356
358	318.0	166.5	171.4	170.5	164.3	245.3	239.8	218.6	296.2	358
360	319.8	167.5	172.5	171.5	165.3	246.7	241.2	219.9	297.8	360
362	321.6	168.5	173.5	172.6	166.4	248.1	242.5	221.2	299.5	362
364	323.3	169.6	174.6	173.7	167.4	249.5	243.9	222.5	301.2	364
366	325.1	170.6	175.6	174.7	168.5	250.9	245.3	223.7	302.8	366
368	326.9	171.6	176.7	175.8	169.5	252.3	246.7	225.0	304.5	368
370	328.7	172.7	177.7	176.8	170.6	253.7	248.1	226.3	306.1	370
372	330.4	173.7	178.8	177.9	171.6	255.1	249.5	227.6	307.8	372
374	332.2	174.7	179.8	179.0	172.7	256.5	250.9	228.9	309.5	374
376	334.0	175.8	180.9	180.0	173.7	257.9	252.2	230.2	311.1	376
378	335.8	176.8	182.0	181.1	174.8	259.3	253.6	231.5	312.8	378
380	337.5	177.9	183.0	182.1	175.9	260.7	255.0	232.8	314.5	380
382	339.3	178.9	184.1	183.2	176.9	262.1	256.4	234.1	316.1	382
384	341.1	180.0	185.2	184.3	178.0	263.5	257.8	235.4	317.8	384
386	342.9	181.0	186.2	185.4	179.1	264.9	259.2	236.6	319.4	386
388	344.6	182.0	187.3	186.4	180.1	266.5	260.5	237.9	321.1	388
390	346.4	183.1	188.4	187.5	181.2	267.7	261.9	239.2	322.8	390
392	348.2	184.1	189.4	188.6	182.3	269.1	263.3	240.5	324.4	392
394	350.0	185.2	190.5	189.7	183.3	270.5	264.7	241.8	326.1	394
396	351.8	186.2	191.6	190.7	184.4	271.9	266.1	243.1	327.7	396
398	353.5	187.3	192.7	191.8	185.5	273.3	267.5	244.4	329.4	398
400	355.3	188.4	193.7	192.9	186.5	274.7	268.9	245.7	331.1	400
402	357.1	189.4	194.8	194.0	187.6	276.1	270.3	247.0	332.7	402
404	358.9	190.5	195.9	195.0	188.7	277.5	271.7	248.3	334.4	404
406	360.6	191.5	197.0	196.1	189.8	278.9	273.0	249.6	336.0	406
408	362.4	192.6	198.1	197.2	190.8	280.3	274.4	251.0	337.7	408

TABLE 1. MUNSON AND WALKER SUGAR TABLE (in milligrams)—*Continued*

CUPROUS OXIDE (Cu_2O)	COPPER (Cu)	DEXTROSE (*d*-GLUCOSE)	INVERT SUGAR	INVERT SUGAR AND SUCROSE		LACTOSE	LACTOSE AND SUCROSE		MALTOSE	CUPROUS OXIDE (Cu_2O)
				0.4 gram total sugar	2 grams total sugar	$C_{12}H_{22}O_{11}+H_2O$	1 lactose, 4 sucrose	1 lactose, 12 sucrose	$C_{12}H_{22}O_{11}+H_2O$	
410	364.2	193.7	199.1	198.3	191.9	281.7	275.8	252.3	339.4	410
412	366.0	194.7	200.2	199.4	193.0	283.2	277.2	253.6	341.0	412
414	367.7	195.8	201.3	200.5	194.1	284.6	278.6	254.9	342.7	414
416	369.5	196.8	202.4	201.6	195.2	286.0	280.0	256.2	344.4	416
418	371.3	197.9	203.5	202.6	196.2	287.4	281.4	257.5	346.0	418
420	373.1	199.0	204.6	203.7	197.3	288.8	282.8	258.8	347.7	420
422	374.8	200.1	205.7	204.8	198.4	290.2	284.2	260.1	349.3	422
424	376.6	201.1	206.7	205.9	199.5	291.6	285.6	261.4	351.0	424
426	378.4	202.2	207.8	207.0	200.6	293.0	287.0	262.7	352.7	426
428	380.2	203.3	208.9	208.1	201.7	294.4	288.4	264.0	354.3	428
430	382.0	204.4	210.0	209.2	202.7	295.8	289.8	265.4	356.0	430
432	383.7	205.5	211.1	210.3	203.8	297.2	291.2	266.6	357.6	432
434	385.5	206.5	212.2	211.4	204.9	298.6	292.6	268.0	359.3	434
436	387.3	207.6	213.3	212.5	206.0	300.0	294.0	269.3	361.0	436
438	389.1	208.7	214.4	213.6	207.1	301.4	295.4	270.6	362.6	438
440	390.8	209.8	215.5	214.7	208.2	302.8	296.8	272.0	364.3	440
442	392.6	210.9	216.6	215.8	209.3	304.2	298.2	273.3	365.9	442
444	394.4	212.0	217.8	216.9	210.4	305.6	299.6	274.6	367.6	444
446	396.2	213.1	218.9	218.0	211.5	307.0	301.0	275.9	369.3	446
448	397.9	214.1	220.0	219.1	212.6	308.4	302.4	277.2	370.9	448
450	399.7	215.2	221.1	220.2	213.7	309.9	303.8	278.6	372.6	450
452	401.5	216.3	222.2	221.4	214.8	311.3	305.2	279.9	374.2	452
454	403.3	217.4	223.3	222.5	215.9	312.7	306.6	281.2	375.9	454
456	405.1	218.5	224.4	223.6	217.0	314.1	308.0	282.5	377.6	456
458	406.8	219.6	225.5	224.7	218.1	315.5	309.4	283.9	379.2	458
460	408.6	220.7	226.7	225.8	219.2	316.9	310.8	285.2	380.9	460
462	410.4	221.8	227.8	226.9	220.3	318.3	312.2	286.5	382.5	462
464	412.2	222.9	228.9	228.1	221.4	319.7	313.6	287.8	384.2	464
466	413.9	224.0	230.0	229.2	222.5	321.1	315.0	289.2	385.9	466
468	415.7	225.1	231.2	230.3	223.7	322.5	316.4	290.5	387.5	468
470	417.5	226.2	232.3	231.4	224.8	323.9	317.7	291.8	389.2	470
472	419.3	227.4	233.4	232.5	225.9	325.3	319.1	293.2	390.8	472
474	421.0	228.3	234.5	233.7	227.0	326.8	320.5	294.5	392.5	474
476	422.8	229.6	235.7	234.8	228.1	328.2	321.9	295.8	394.2	476
478	424.6	230.7	236.8	235.9	229.2	329.6	323.3	297.1	395.8	478
480	426.4	231.8	237.9	237.1	230.3	331.0	324.7	298.5	397.5	480
482	428.1	232.9	239.1	238.2	231.5	332.4	326.1	299.8	399.1	482
484	429.9	234.1	240.2	239.3	232.6	333.8	327.5	301.1	400.8	484
486	431.7	235.2	241.4	240.5	233.7	335.2	328.9	302.5	402.4	486
488	433.5	236.3	242.5	241.6	234.8	336.6	330.3	303.8	404.1	488
490	435.3	237.4	243.6	242.7	236.0	338.0	331.7	305.1	405.8	490

TABLE 2. LANE-EYNON SUGAR TABLE—[1] (INVERT SUGAR)

(10 cc. of Fehling's solution)

Cc. of sugar solution required	Solutions containing besides invert sugar:					
	No sucrose		1 g. sucrose per 100 cc.		5 g. sucrose per 100 cc.	
	Invert sugar factor[1]	Mg. invert sugar per 100 cc.	Invert sugar factor[1]	Mg. invert sugar per 100 cc.	Invert sugar factor[1]	Mg. invert sugar per 100 cc.
15	50.5	336	49.9	333	47.6	317
16	50.6	316	50.0	312	47.6	297
17	50.7	298	50.1	295	47.6	280
18	50.8	282	50.1	278	47.6	264
19	50.8	267	50.2	264	47.6	250
20	50.9	254.5	50.2	251.0	47.6	238.0
21	51.0	242.9	50.2	239.0	47.6	226.7
22	51.0	231.8	50.3	228.2	47.6	216.4
23	51.1	222.2	50.3	218.7	47.6	207.0
24	51.2	213.3	50.3	209.8	47.6	198.3
25	51.2	204.8	50.4	201.6	47.6	190.4
26	51.3	197.4	50.4	193.8	47.6	183.1
27	51.4	190.4	50.4	186.7	47.6	176.4
28	51.4	183.7	50.5	180.2	47.7	170.3
29	51.5	177.6	50.5	174.1	47.7	164.5
30	51.5	171.7	50.5	168.3	47.7	159.0
31	51.6	166.3	50.6	163.1	47.7	153.9
32	51.6	161.2	50.6	158.1	47.7	149.1
33	51.7	156.6	50.6	153.3	47.7	144.5
34	51.7	152.2	50.6	148.9	47.7	140.3
35	51.8	147.9	50.7	144.7	47.7	136.3
36	51.8	143.9	50.7	140.7	47.7	132.5
37	51.9	140.2	50.7	137.0	47.7	128.9
38	51.9	136.6	50.7	133.5	47.7	125.5
39	52.0	133.3	50.8	130.2	47.7	122.3
40	52.0	130.1	50.8	127.0	47.7	119.2
41	52.1	127.1	50.8	123.9	47.7	116.3
42	52.1	124.2	50.8	121.0	47.7	113.5
43	52.2	121.4	50.8	118.2	47.7	110.9
44	52.2	118.7	50.9	115.6	47.7	108.4
45	52.3	116.1	50.9	113.1	47.7	106.0
46	52.3	113.7	50.9	110.6	47.7	103.7
47	52.4	111.4	50.9	108.2	47.7	101.5
48	52.4	109.2	50.9	106.0	47.7	99.4
49	52.5	107.1	51.0	104.0	47.7	97.4
50	52.5	105.1	51.0	102.0	47.7	95.4

[1] Lane and Eynon, *J. Soc. Chem. Ind.* **42**, 32T (1923).

[2] Mg. of invert sugar corresponding to 10 cc. of Fehling's solution.

Table 2. Lane-Eynon Sugar Table[3] (Dextrose[1] and Levulose[2])

(10 cc. of Fehling's solution.)

Cc. of sugar solution required	Dextrose[1] factor	Mg. dextrose per 100 cc.[4]	Levulose[5] factor	Mg. levulose per 100 cc.
15	49.1	327	52.2	348
16	49.2	307	52.3	327
17	49.3	289	52.3	308
18	49.3	274	52.4	291
19	49.4	260	52.5	276
20	49.5	247.4	52.5	262.5
21	49.5	235.8	52.6	250.6
22	49.6	225.5	52.7	239.6
23	49.7	216.1	52.7	229.1
24	49.8	207.4	52.8	220.0
25	49.8	199.3	52.8	211.3
26	49.9	191.8	52.9	203.3
27	49.9	184.9	52.9	196.0
28	50.0	178.5	53.0	189.3
29	50.0	172.5	53.1	183.1
30	50.1	167.0	53.2	177.2
31	50.2	161.8	53.2	171.7
32	50.2	156.9	53.3	166.5
33	50.3	152.4	53.3	161.6
34	50.3	148.0	53.4	157.0
35	50.4	143.9	53.4	152.6
36	50.4	140.0	53.5	148.6
37	50.5	136.4	53.5	144.7
38	50.5	132.9	53.6	140.9
39	50.6	129.6	53.6	137.3
40	50.6	126.5	53.6	134.0
41	50.7	123.6	53.7	130.9
42	50.7	120.8	53.7	127.9
43	50.8	118.1	53.8	125.1
44	50.8	115.5	53.8	122.4
45	50.9	113.0	53.9	119.8
46	50.9	110.6	53.9	117.2
47	51.0	108.4	53.9	114.7
48	51.0	106.2	54.0	112.4
49	51.0	104.1	54.0	110.2
50	51.1	102.2	54.0	108.0

[1] All figures relate to anhydrous dextrose.

[2] All figures relate to anhydrous levulose.

[3] Lane and Eynon, *J. Soc. Chem. Ind.* **42**, 32T (1923).

[4] Mg. dextrose corresponding to 10 cc. of Fehling's solution.

[5] Mg. levulose corresponding to 10 cc. of Fehling's solution.

TABLE 2. LANE-EYNON SUGAR TABLE[1] (LACTOSE)

(10 cc. of Fehling's solution.)

Cc. of sugar solution required	Hydrated lactose $C_{12}H_{22}O_{11}.H_2O$		Anhydrous lactose $C_{12}H_{22}O_{11}$	
	Factor[2]	Mg. per 100 cc.	Factor[2]	Mg. per 100 cc.
15	68.3	455	64.9	432
16	68.2	426	64.8	405
17	68.2	401	64.8	381
18	68.1	378	64.7	359
19	68.1	358	64.7	340
20	68.0	340.0	64.6	323.0
21	68.0	323.8	64.6	307.6
22	68.0	309.1	64.6	293.6
23	67.9	295.4	64.5	280.6
24	67.9	282.9	64.5	268.8
25	67.9	271.6	64.5	258.0
26	67.9	261.0	64.5	248.0
27	67.8	251.1	64.4	238.5
28	67.8	242.1	64.4	230.0
29	67.8	233.8	64.4	222.2
30	67.8	226.0	64.4	214.7
31	67.8	218.7	64.4	207.8
32	67.8	211.9	64.4	201.3
33	67.8	205.6	64.4	195.3
34	67.9	199.7	64.5	189.7
35	67.9	194.0	64.5	184.3
36	67.9	188.6	64.5	179.2
37	67.9	183.5	64.5	174.3
38	67.9	178.7	64.5	169.8
39	67.9	174.1	64.5	165.4
40	67.9	169.7	64.5	161.2
41	68.0	165.9	64.6	157.6
42	68.0	161.9	64.6	153.8
43	68.0	158.1	64.6	150.2
44	68.0	154.7	64.6	147.0
45	68.1	151.3	64.7	143.7
46	68.1	148.0	64.7	140.6
47	68.2	145.1	64.8	137.8
48	68.2	142.1	64.8	135.0
49	68.2	139.2	64.8	132.2
50	68.3	136.6	64.9	129.8

[1] Lane and Eynon, *J. Soc. Chem. Ind.* 42, 32T (1923).

[2] Mg. lactose corresponding to 10 cc. of Fehling's solution.

TABLE 2. LANE-EYNON SUGAR TABLE[1] (MALTOSE)

(10 cc. of Fehling's solution.)

Cc. of sugar solution required	Hydrated maltose $C_{12}H_{22}O_{11}.H_2O$		Anhydrous maltose $C_{12}H_{22}O_{11}$	
	Factor[2]	Mg. per 100 cc.	Factor[2]	Mg. per 100 cc.
15	81.3	542	77.2	515
16	81.2	507	77.1	482
17	81.1	477	77.0	453
18	81.0	450	77.0	427
19	80.9	426	76.9	405
20	80.8	404.0	76.8	383.8
21	80.7	384.3	76.7	365.1
22	80.6	366.4	76.6	348.1
23	80.5	350.0	76.5	332.5
24	80.4	335.0	76.4	318.3
25	80.4	321.5	76.4	305.4
26	80.3	308.8	76.3	293.4
27	80.2	297.0	76.2	282.2
28	80.1	286.1	76.1	271.8
29	80.0	276.0	76.0	262.2
30	80.0	266.6	76.0	253.3
31	79.9	257.8	75.9	244.9
32	79.9	249.7	75.9	237.2
33	79.8	241.9	75.8	229.8
34	79.8	234.6	75.8	222.9
35	79.7	227.6	75.7	216.2
36	79.6	221.1	75.6	210.0
37	79.6	215.0	75.6	204.3
38	79.5	209.2	75.5	198.7
39	79.5	203.8	75.5	193.6
40	79.4	198.5	75.4	188.6
41	79.4	193.7	75.4	184.3
42	79.3	188.8	75.3	179.4
43	79.3	184.3	75.3	175.1
44	79.2	180.0	75.2	171.0
45	79.2	175.9	75.2	167.1
46	79.1	172.0	75.1	163.4
47	79.1	168.3	75.1	159.9
48	79.1	164.7	75.1	156.5
49	79.0	161.2	75.0	153.1
50	79.0	158.0	75.0	150.1

[1] Lane and Eynon, *J. Soc. Chem. Ind.* **42**, 32T (1923).

[2] Mg. of maltose corresponding to 10 cc. of Fehling's solution.

TABLE 3. SPECIFIC GRAVITY AND REFRACTIVE INDEX OF SUGAR SOLUTIONS[1,2]

Degrees Brix or per cent by weight of sucrose	Specific gravity at 20/20°C.	Specific gravity at 20/4°C.	Degrees Baumé (modulus 145)	Refractive index at 20°	Degrees Brix or per cent by weight of sucrose	Specific gravity at 20/20°C.	Specific gravity at 20/4°C.	Degrees Baumé (modulus 145)	Refractive index at 20°
0.0	1.00000	0.9982	0.00	1.3330	9.0	1.03586	1.0340	5.02	1.3464
0.2	.00078	0.9990	0.11	.3333	9.2	.03668	.0348	5.13	.3467
0.4	.00155	0.9998	0.22	.3336	9.4	.03750	.0357	5.24	.3470
0.6	.00233	1.0006	0.34	.3338	9.6	.03833	.0365	5.35	.3473
0.8	.00311	.0013	0.45	.3341	9.8	.03915	.0373	5.46	.3476
1.0	.00389	.0021	0.56	.3344	10.0	.03998	.0381	5.57	.3479
1.2	.00467	.0029	0.67	.3347	10.2	.04081	.0390	5.68	.3482
1.4	.00545	.0037	0.79	.3350	10.4	.04164	.0398	5.80	.3485
1.6	.00623	.0045	0.90	.3353	10.6	.04247	.0406	5.91	.3488
1.8	.00701	.0052	1.01	.3356	10.8	.04330	.0415	6.02	.3491
2.0	.00779	.0060	1.12	.3359	11.0	.04413	.0423	6.13	.3494
2.2	.00858	.0068	1.23	.3362	11.2	.04497	.0431	6.24	.3497
2.4	.00936	.0076	1.34	.3365	11.4	.04580	.0440	6.35	.3500
2.6	.01015	.0084	1.46	.3368	11.6	.04664	.0448	6.46	.3504
2.8	.01093	.0091	1.57	.3371	11.8	.04747	.0456	6.57	.3507
3.0	.01172	.0099	1.68	.3373	12.0	.04831	.0465	6.68	.3510
3.2	.01251	.0107	1.79	.3377	12.2	.04915	.0473	6.79	.3513
3.4	.01330	.0115	1.90	.3380	12.4	.04999	.0481	6.90	.3516
3.6	.01409	.0123	2.02	.3382	12.6	.05084	.0490	7.02	.3520
3.8	.01488	.0131	2.13	.3385	12.8	.05168	.0498	7.13	.3523
4.0	.01567	.0139	2.24	.3388	13.0	.05252	.0507	7.24	.3526
4.2	.01647	.0147	2.35	.3391	13.2	.05337	.0515	7.35	.3529
4.4	.01726	.0155	2.46	.3394	13.4	.05422	.0524	7.46	.3532
4.6	.01806	.0163	2.57	.3397	13.6	.05506	.0532	7.57	.3535
4.8	.01886	.0171	2.68	.3400	13.8	.05591	.0540	7.68	.3538
5.0	.01965	.0179	2.79	.3403	14.0	.05677	.0549	7.79	.3541
5.2	.02045	.0187	2.91	.3406	14.2	.05762	.0558	7.90	.3544
5.4	.02125	.0195	3.02	.3409	14.4	.05847	.0566	8.01	.3547
5.6	.02206	.0203	3.13	.3412	14.6	.05933	.0575	8.12	.3551
5.8	.02286	.0211	3.24	.3415	14.8	.06018	.0583	8.23	.3554
6.0	.02366	.0219	3.35	.3418	15.0	.06104	.0592	8.34	.3557
6.2	.02447	.0227	3.46	.3421	15.2	.06190	.0600	8.45	.3560
6.4	.02527	.0235	3.57	.3424	15.4	.06276	.0609	8.56	.3563
6.6	.02608	.0243	3.69	.3427	15.6	.06362	.0617	8.67	.3567
6.8	.02689	.0251	3.80	.3430	15.8	.06448	.0626	8.78	.3570
7.0	.02770	.0259	3.91	.3433	16.0	.06534	.0635	8.89	.3573
7.2	.02851	.0267	4.02	.3436	16.2	.06621	.0643	9.00	.3576
7.4	.02932	.0275	4.13	.3439	16.4	.06707	.0652	9.11	.3580
7.6	.03013	.0283	4.24	.3442	16.6	.06794	.0661	9.22	.3583
7.8	.03095	.0291	4.35	.3445	16.8	.06881	.0669	9.33	.3587
8.0	.03176	.0299	4.46	.3448	17.0	.06968	.0678	9.45	.3590
8.2	.03258	.0308	4.58	.3451	17.2	.07055	.0687	9.56	.3593
8.4	.03340	.0316	4.69	.3454	17.4	.07142	.0695	9.67	.3596
8.6	.03422	.0324	4.80	.3458	17.6	.07229	.0704	9.78	.3600
8.8	.03504	.0332	4.91	.3461	17.8	.07317	.0713	9.89	.3603

[1] U. S. Bureau of Standards, Circ. No. 19 (1924).

[2] Report International Commission Uniform Methods Sugar Analysis, Analyst, 62, 197 (1937).

Table 3. Specific Gravity and Refractive Index of Sugar Solutions —*Continued*

Degrees Brix or per cent by weight of sucrose	Specific gravity at 20/20°C	Specific gravity at 20/4°C	Degrees Baumé (modulus 145)	Refractive index at 20°	Degrees Brix or per cent by weight of sucrose	Specific gravity at 20/20°C	Specific gravity at 20/4°C	Degrees Baumé (modulus 145)	Refractive index at 20°
18.0	1.07404	1.0721	10.00	1.3606	27.0	1.11480	1.1128	14.93	1.3758
18.2	.07492	.0730	10.11	.3609	27.2	.11573	.1138	15.04	.3761
18.4	.07580	.0739	10.22	.3612	27.4	.11667	.1147	15.15	.3765
18.6	.07668	.0748	10.33	.3616	27.6	.11761	.1156	15.26	.3768
18.8	.07756	.0757	10.44	.3619	27.8	.11855	.1166	15.37	.3772
19.0	.07844	.0765	10.55	.3622	28.0	.11949	.1175	15.48	.3775
19.2	.07932	.0774	10.66	.3625	28.2	.12043	.1185	15.59	.3779
19.4	.08021	.0783	10.77	.3629	28.4	.12138	.1194	15.69	.3782
19.6	.08110	.0792	10.88	.3632	28.6	.12232	.1203	15.80	.3886
19.8	.08198	.0801	10.99	.3636	28.8	.12327	.1213	15.91	.3789
20.0	.08287	.0810	11.10	.3639	29.0	.12422	.1222	16.02	.3793
20.2	.08376	.0818	11.21	.3642	29.2	.12517	.1232	16.13	.3797
20.4	.08465	.0827	11.32	.3645	29.4	.12612	.1241	16.24	.3800
20.6	.08554	.0836	11.43	.3649	29.6	.12707	.1251	16.35	.3804
20.8	.08644	.0845	11.54	.3652	29.8	.12802	.1260	16.46	.3807
21.0	.08733	.0854	11.65	.3655	30.0	.12898	.1270	16.57	.3811
21.2	.08823	.0863	11.76	.3658	30.2	.12993	.1279	16.67	.3815
21.4	.08913	.0872	11.87	.3662	30.4	.13089	.1289	16.78	.3818
21.6	.09003	.0881	11.98	.3665	30.6	.13185	.1299	16.89	.3822
21.8	.09093	.0890	12.09	.3669	30.8	.13281	.1308	17.00	.3825
22.0	.09183	.0899	12.20	.3672	31.0	.13378	.1318	17.11	.3829
22.2	.09273	.0908	12.31	.3675	31.2	.13474	.1327	17.22	.3833
22.4	.09364	.0917	12.42	.3679	31.4	.13570	.1337	17.33	.3836
22.6	.09454	.0926	12.52	.3682	31.6	.13667	.1347	17.43	.3840
22.8	.09545	.0935	12.63	.3686	31.8	.13764	.1356	17.54	.3843
23.0	.09636	.0944	12.74	.3689	32.0	.13861	.1366	17.65	.3847
23.2	.09727	.0953	12.85	.3692	32.2	.13958	.1376	17.76	.3851
23.4	.09818	.0962	12.96	.3696	32.4	.14055	.1385	17.87	.3854
23.6	.09909	.0971	13.07	.3699	32.6	.14152	.1395	17.98	.3858
23.8	.10000	.0981	13.18	.3703	32.8	.14250	.1405	18.08	.3861
24.0	.10092	.0990	13.29	.3706	33.0	.14347	.1415	18.19	.3865
24.2	.10183	.0999	13.40	.3709	33.2	.14445	.1424	18.30	.3869
24.4	.10275	.1008	13.51	.3713	33.4	.14543	.1434	18.41	.3872
24.6	.10367	.1017	13.62	.3716	33.6	.14641	.1444	18.52	.3876
24.8	.10459	.1026	13.73	.3720	33.8	.14739	.1454	18.63	.3879
25.0	.10551	.1036	13.84	.3723	34.0	.14837	.1463	18.73	.3883
25.2	.10643	.1045	13.95	.3726	34.2	.14936	.1473	18.84	.3887
25.4	.10736	.1054	14.06	.3730	34.4	.15034	.1483	18.95	.3891
25.6	.10828	.1063	14.17	.3733	34.6	.15133	.1493	19.06	.3894
25.8	.10921	.1072	14.28	.3737	34.8	.15232	.1503	19.17	.3898
26.0	.11014	.1082	14.39	.3740	35.0	.15331	.1513	19.28	.3902
26.2	.11106	.1091	14.49	.3744	35.2	.15430	.1523	19.38	.3906
26.4	.11200	.1100	14.60	.3747	35.4	.15530	.1533	19.49	.3909
26.6	.11293	.1111	14.71	.3751	35.6	.15629	.1542	19.60	.3913
26.8	.11386	.1110	14.82	.3754	35.8	.15729	.1552	19.71	.3916

TABLE 3. SPECIFIC GRAVITY AND REFRACTIVE INDEX OF SUGAR SOLUTIONS —*Continued*

Degrees Brix or per cent by weight of sucrose	Specific gravity at 20/20°C.	Specific gravity at 20/4°C.	Degrees Baumé (modulus 145)	Refractive index at 20°	Degrees Brix or per cent by weight of sucrose	Specific gravity at 20/20°C.	Specific gravity at 20/4°C.	Degrees Baumé (modulus 145)	Refractive index at 20°
36.0	1.15828	1.1562	19.81	1.3920	45.0	1.20467	1.2025	24.63	1.4096
36.2	.15928	.1572	19.92	.3924	45.2	.20573	.2036	24.74	.4100
36.4	.16028	.1582	20.03	.3928	45.4	.20680	.2047	24.85	.4104
36.6	.16128	.1592	20.14	.3931	45.6	.20787	.2057	24.95	.4109
36.8	.16228	.1602	20.25	.3935	45.8	.20894	.2068	25.06	.4113
37.0	.16329	.1612	20.35	.3939	46.0	.21001	.2079	25.17	.4117
37.2	.16430	.1622	20.46	.3943	46.2	.21108	.2089	25.27	.4121
37.4	.16530	.1632	20.57	.3947	46.4	.21215	.2100	25.38	.4125
37.6	.16631	.1643	20.68	.3950	46.6	.21323	.2111	25.48	.4129
37.8	.16732	.1653	20.78	.3954	46.8	.21431	.2122	25.59	.4133
38.0	.16833	.1663	20.89	.3958	47.0	.21538	.2132	25.70	.4137
38.2	.16934	.1673	21.00	.3962	47.2	.21646	.2143	25.80	.4141
38.4	.17036	.1683	21.11	.3966	47.4	.21755	.2154	25.91	.4145
38.6	.17138	.1693	21.21	.3970	47.6	.21863	.2165	26.01	.4150
38.8	.17239	.1703	21.32	.3974	47.8	.21971	.2176	26.12	.4154
39.0	.17341	.1713	21.43	.3978	48.0	.22080	.2186	26.23	.4158
39.2	.17443	.1724	21.54	.3982	48.2	.22189	.2197	26.33	.4162
39.4	.17545	.1734	21.64	.3986	48.4	.22298	.2208	26.44	.4166
39.6	.17648	.1744	21.75	.3989	48.6	.22406	.2219	26.54	.4171
39.8	.17750	.1754	21.86	.3993	48.8	.22516	.2230	26.65	.4175
40.0	.17853	.1764	21.97	.3997	49.0	.22625	.2241	26.75	.4179
40.2	.17956	.1775	22.07	.4001	49.2	.22735	.2252	26.86	.4183
40.4	.18058	.1785	22.18	.4005	49.4	.22844	.2263	26.96	.4187
40.6	.18162	.1795	22.29	.4008	49.6	.22954	.2274	27.07	.4192
40.8	.18265	.1806	22.39	.4012	49.8	.23064	.2285	27.18	.4196
41.0	.18368	.1816	22.50	.4016	50.0	.23174	.2296	27.28	.4200
41.2	.18472	.1826	22.61	.4020	50.2	.23284	.2307	27.39	.4204
41.4	.18575	.1837	22.72	.4024	50.4	.23395	.2318	27.49	.4208
41.6	.18679	.1847	22.82	.4028	50.6	.23506	.2329	27.60	.4213
41.8	.18783	.1857	22.93	.4032	50.8	.23616	.2340	27.70	.4217
42.0	.18887	.1868	23.04	.4036	51.0	.23727	.2351	27.81	.4221
42.2	.18992	.1878	23.14	.4040	51.2	.23838	.2362	27.91	.4225
42.4	.19096	.1889	23.25	.4044	51.4	.23949	.2373	28.02	.4229
42.6	.19201	.1899	23.36	.4048	51.6	.24060	.2384	28.12	.4234
42.8	.19305	.1909	23.46	.4052	51.8	.24172	.2395	28.23	.4238
43.0	.19410	.1920	23.57	.4056	52.0	.24284	.2406	28.33	.4242
43.2	.19515	.1930	23.68	.4060	52.2	.24395	.2418	28.44	.4246
43.4	.19620	.1941	23.78	.4064	52.4	.24507	.2429	28.54	.4251
43.6	.19726	.1951	23.89	.4068	52.6	.24619	.2440	28.65	.4255
43.8	.19831	.1962	24.00	.4072	52.8	.24731	.2451	28.75	.4260
44.0	.19936	.1972	24.10	.4076	53.0	.24844	.2462	28.86	.4264
44.2	.20042	.1983	24.21	.4080	53.2	.24956	.2474	28.96	.4268
44.4	.20148	.1994	24.32	.4084	53.4	.25069	.2485	29.06	.4272
44.6	.20254	.2004	24.42	.4088	53.6	.25182	.2496	29.17	.4277
44.8	.20360	.2015	24.53	.4092	53.8	.25295	.2507	29.27	.4281

Table 3. Specific Gravity and Refractive Index of Sugar Solutions —*Continued*

Degrees Brix or per cent by weight of sucrose	Specific gravity at 20/20°C.	Specific gravity at 20/4°C.	Degrees Baumé (modulus 145)	Refractive index at 20°	Degrees Brix or per cent by weight of sucrose	Specific gravity at 20/20°C.	Specific gravity at 20/4°C.	Degrees Baumé (modulus 145)	Refractive index at 20°
54.0	1.25408	1.2519	29.38	1.4285	63.0	1.30657	1.3043	34.02	1.4486
54.2	.25521	.2530	29.48	.4289	63.2	.30778	.3055	34.12	.4491
54.4	.25635	.2541	29.59	.4294	63.4	.30898	.3067	34.23	.4495
54.6	.25748	.2553	29.69	.4298	63.6	.31019	.3079	34.33	.4500
54.8	.25862	.2564	29.80	.4303	63.8	.31139	.3091	34.43	.4504
55.0	.25976	.2575	29.90	.4307	64.0	.31260	.3103	34.53	.4509
55.2	.26090	.2587	30.00	.4311	64.2	.31381	.3115	34.63	.4514
55.4	.26204	.2598	30.11	.4316	64.4	.31502	.3127	34.74	.4518
55.6	.26319	.2610	30.21	.4320	64.6	.31623	.3139	34.84	.4523
55.8	.26433	.2621	30.32	.4325	64.8	.31745	.3151	34.94	.4527
56.0	.26548	.2632	30.42	.4329	65.0	.31866	.3163	35.04	.4532
56.2	.26663	.2644	30.52	.4333	65.2	.31988	.3175	35.14	.4537
56.4	.26778	.2655	30.63	.4338	65.4	.32110	.3188	35.24	.4541
56.6	.26893	.2667	30.73	.4342	65.6	.32232	.3200	35.34	.4546
56.8	.27008	.2678	30.83	.4347	65.8	.32354	.3212	35.45	.4550
57.0	.27123	.2690	30.94	.4351	66.0	.32476	.3224	35.55	.4555
57.2	.27239	.2701	31.04	.4355	66.2	.32599	.3236	35.65	.4560
57.4	.27355	.2713	31.15	.4360	66.4	.32722	.3249	35.75	.4565
57.6	.27471	.2725	31.25	.4364	66.6	.32844	.3261	35.85	.4569
57.8	.27587	.2736	31.35	.4369	66.8	.32967	.3273	35.95	.4574
58.0	.27703	.2748	31.46	.4373	67.0	.33090	.3286	36.05	.4579
58.2	.27819	.2759	31.56	.4378	67.2	.33214	.3298	36.15	.4584
58.4	.27936	.2771	31.66	.4382	67.4	.33337	.3310	36.25	.4589
58.6	.28052	.2783	31.76	.4387	67.6	.33460	.3322	36.35	.4593
58.8	.28169	.2794	31.87	.4391	67.8	.33584	.3335	36.45	.4598
59.0	.28286	.2806	31.97	.4396	68.0	.33708	.3347	36.55	.4603
59.2	.28404	.2818	32.07	.4400	68.2	.33832	.3360	36.66	.4607
59.4	.28520	.2829	32.18	.4405	68.4	.33957	.3372	36.76	.4612
59.6	.28638	.2841	32.28	.4409	68.6	.34081	.3384	36.86	.4617
59.8	.28755	.2853	32.38	.4414	68.8	.34205	.3397	36.96	.4622
60.0	.28873	.2865	32.49	.4418	69.0	.34330	.3409	37.06	.4627
60.2	.28991	.2876	32.59	.4423	69.2	.34455	.3422	37.16	.4631
60.4	.29109	.2888	32.69	.4427	69.4	.34580	.3434	37.26	.4636
60.6	.29227	.2900	32.79	.4432	69.6	.34705	.3447	37.36	.4641
60.8	.29346	.2912	32.90	.4436	69.8	.34830	.3459	37.46	.4646
61.0	.29464	.2924	33.00	.4441	70.0	.34956	.3472	37.56	.4651
61.2	.29583	.2935	33.10	.4446	70.2	.35081	.3484	37.66	.4656
61.4	.29701	.2947	33.20	.4450	70.4	.35207	.3497	37.76	.4661
61.6	.29820	.2959	33.31	.4455	70.6	.35333	.3509	37.86	.4666
61.8	.29940	.2971	33.41	.4459	70.8	.35459	.3522	37.96	.4671
62.0	.30059	.2983	33.51	.4464	71.0	.35585	.3535	38.06	.4676
62.2	.30178	.2995	33.61	.4468	71.2	.35711	.3547	38.16	.4681
62.4	.30298	.3007	33.72	.4473	71.4	.35838	.3560	38.26	.4685
62.6	.30418	.3019	33.82	.4477	71.6	.35964	.3572	38.35	.4690
62.8	.30537	.3031	33.92	.4482	71.8	.36091	.3585	38.45	.4695

TABLE 3. SPECIFIC GRAVITY AND REFRACTIVE INDEX OF SUGAR SOLUTIONS —*Continued*

Degrees Brix or per cent by weight of sucrose	Specific gravity at 20/20°C.	Specific gravity at 20/4°C.	Degrees Baumé (modulus 145)	Refractive index at 20°	Degrees Brix or per cent by weight of sucrose	Specific gravity at 20/20°C.	Specific gravity at 20/4°C.	Degrees Baumé (modulus 145)	Refractive index at 20°
72.0	1.36218	1.3598	38.55	1.4700	81.0	1.42088	1.4184	42.95	1.4927
72.2	.36346	.3610	38.65	.4705	81.2	.42222	.4197	43.05	.4933
72.4	.36473	.3623	38.75	.4710	81.4	.42356	.4210	43.14	.4938
72.6	.36600	.3636	38.85	.4715	81.6	.42490	.4224	43 24	.4943
72.8	.36728	.3649	38.95	.4720	81.8	.42625	.4237	43.33	.4949
73.0	.36856	.3661	39.05	.4725	82.0	.42759	.4251	43.43	.4954
73.2	.36983	.3674	39.15	.4730	82.2	.42894	.4264	43.53	.4959
73.4	.37111	.3687	39.25	.4735	82.4	43029	.4278	43.62	.4964
73.6	.37240	.3700	39.35	.4740	82.6	.43164	.4291	43.72	.4970
73.8	.37368	.3713	39.44	.4744	82.8	.43298	.4305	43.81	.4975
74.0	.37496	.3725	39.54	.4749	83.0	.43434	.4318	43.91	.4980
74.2	.37625	.3738	39.64	.4754	83.2	43569	.4332	44.00	.4985
74.4	.37754	.3751	39.74	.4759	83.4	.43705	.4345	44.10	.4991
74.6	.37883	.3764	39.84	.4764	83.6	.43841	4359	44.19	.4996
74.8	.38012	.3777	39.94	.4769	83.8	.43976	.4372	44.29	.5001
75.0	.38141	.3790	40.03	.4774	84.0	.44112	.4386	44.38	.5007
75.2	.38270	.3803	40.13	.4779	84.2	.44249	.4399	44.48	.5012
75.4	.38400	.3816	40.23	.4784	84.4	.44385	.4413	44.57	.5017
75.6	.38530	.3829	40.33	.4789	84.6	.44521	.4427	44.67	.5022
75.8	.38660	.3841	40.43	.4794	84.8	.44658	.4440	44.76	.5028
76.0	.38790	.3854	40.53	.4799	85.0	.44794	.4454	44.86	.5033
76.2	.38920	.3867	40.62	.4804	85.2	.44931	4468	44.95	
76.4	.39050	.3880	40.72	.4810	85.4	.45068	.4481	45.05	
76.6	.39180	.3893	40.82	.4815	85.6	.45205	.4495	45.14	
76.8	.39311	.3907	40.92	.4820	85.8	.45343	.4509	45.24	
77.0	.39442	.3920	41.01	.4825	86.0	.45480	4522	45.33	
77.2	.39573	.3933	41.11	.4830	86.2	.45618	4536	45.42	
77.4	.39704	.3946	41.21	.4835	86.4	.45755	4550	45.52	
77.6	.39835	.3959	41.31	.4840	86.6	.45893	.4564	45.61	
77.8	.39966	.3972	41.40	.4845	86.8	.46031	4577	45.71	
78.0	.40098	.3985	41.50	.4850	87.0	.46170	.4591	45.80	
78.2	.40230	.3998	41.60	.4855	87.2	.46308	4605	45.89	
78.4	.40361	.4011	41.70	.4860	87.4	.46446	4619	45.99	
78.6	.40493	.4025	41.79	.4865	87.6	46585	4633	46.08	
78.8	.40625	.4038	41 89	.4871	87.8	.46724	4646	46.17	
79.0	.40758	.4051	41.99	.4876	88 0	.46862	4660	46.27	
79.2	.40890	.4064	42.08	.4881	88.2	.47002	4674	46.36	
79.4	.41023	4077	42.18	.4886	88.4	.47141	4688	46.45	
79.6	.41155	.4091	42.28	.4891	88.6	47280	4702	46.55	
79.8	.41288	.4104	42.37	.4896	88.8	47420	4716	46.64	
80.0	.41421	.4117	42.47	.4901	89 0	.47559	4730	46 73	
80.2	.41554	.4130	42.57	.4906	89.2	.47699	4744	46.83	
80.4	.41688	.4144	42.66	.4912	89.4	.47839	.4758	46.92	
80.6	.41821	.4157	42.76	.4917	89.6	.47979	.4772	47.01	
80.8	.41955	.4170	42.85	.4922	89.8	.48119	.4786	47.11	

TABLE 3. SPECIFIC GRAVITY OF SUGAR SOLUTIONS—*Continued*

Degrees Brix or Per Cent by Weight of Sucrose	Specific Gravity at 20/20°C.	Specific Gravity at 20/4°C.	Degrees Baumé (Modulus 145)	Degrees Brix or Per Cent by Weight of Sucrose	Specific Gravity at 20/20°C.	Specific Gravity at 20/4°C.	Degrees Baumé (Modulus 145)
90.0	1.48259	1.4800	47.20	95.0	1.51814	1.5155	49.49
90.2	.48400	.4814	47.29	95.2	.51958	.5169	49.58
90.4	.48540	.4828	47.38	95.4	.52102	.5183	49.67
90.6	.48681	.4842	47.48	95.6	.52246	.5198	49.76
90.8	.48822	.4856	47.57	95.8	.52390	.5212	49.85
91.0	.48963	.4870	47.66	96.0	.52535	.5227	49.94
91.2	.49104	.4884	47.75	96.2	.52680	.5241	50.03
91.4	.49246	.4898	47.84	96.4	.52824	.5255	50.12
91.6	.49387	.4912	47.94	96.6	.52969	.5270	50.21
91.8	.49529	.4926	48.03	96.8	.53114	.5284	50.30
92.0	.49671	.4941	48.12	97.0	.53260	.5299	50.39
92.2	.49812	.4955	48.21	97.2	.53405	.5313	50.48
92.4	.49954	.4969	48.30	97.4	.53551	.5328	50.57
92.6	.50097	.4983	48.40	97.6	.53696	.5342	50.66
92.8	.50239	.4997	48.49	97.8	.53842	.5357	50.75
93.0	.50381	.5012	48.58	98.0	.53988	.5372	50.84
93.2	.50524	.5026	48.67	98.2	.54134	.5386	50.93
93.4	.50667	.5040	48.76	98.4	.54280	.5401	51.02
93.6	.50810	.5054	48.85	98.6	.54426	.5415	51.10
93.8	.50952	.5069	48.94	98.8	.54573	.5430	51.19
94.0	.51096	.5083	49.03	99.0	.54719	.5445	51.28
94.2	.51239	.5097	49.12	99.2	.54866	.5459	51.37
94.4	.51382	.5112	49.22	99.4	.55013	.5474	51.46
94.6	.51526	.5126	49.31	99.6	.55160	.5489	51.55
94.8	.51670	.5140	49.40	99.8	.55307	.5503	51.64
				100.0	.55454	.5518	51.73

Table 4. Ethyl Alcohol [1]

Specific gravity of mixtures of ethyl alcohol and water.

Apparent specific gravity 20°C/20°C.	Alcohol by volume per cent	Alcohol by weight per cent	Scale reading immersion refractometer at 20°C.	Index of refraction 20°C.
1.0000	0.00	0.00	....	....
0.9998	0.10	0.08	....	....
0.9997	0.20	0.16	....	....
0.9996	0.30	0.24	....	....
0.9994	0.40	0.32	....	....
0.9992	0.50	0.40	15.0	1.33319
0.9991	0.60	0.48	15.1	1.33323
0.9990	0.70	0.56	15.2	1.33327
0.9988	0.80	0.64	15.3	1.33331
0.9987	0.90	0.71	15.4	1.33335
0.9985	1.00	0.79	15.6	1.33343
0.9984	1.10	0.87	15.7	1.33347
0.9982	1.20	0.95	15.8	1.33350
0.9981	1.30	1.03	15.9	1.33354
0.9979	1.40	1.11	16.0	1.33358
0.9978	1.50	1.19	16.2	1.33366
0.9976	1.60	1.27	16.3	1.33370
0.9975	1.70	1.35	16.4	1.33374
0.9973	1.80	1.43	16.6	1.33381
0.9972	1.90	1.51	16.7	1.33385
0.9970	2.00	1.59	16.8	1.33389
0.9969	2.10	1.67	17.0	1.33397
0.9968	2.20	1.75	17.1	1.33401
0.9966	2.30	1.83	17.2	1.33405
0.9965	2.40	1.91	17.4	1.33412
0.9963	2.50	1.99	17.5	1.33416
0.9962	2.60	2.07	17.6	1.33420
0.9960	2.70	2.15	17.8	1.33428
0.9959	2.80	2.23	17.9	1.33432
0.9957	2.90	2.31	18.0	1.33435
0.9956	3.00	2.39	18.2	1.33443
0.9955	3.10	2.47	18.3	1.33447
0.9953	3.20	2.55	18.4	1.33451
0.9952	3.30	2.64	18.6	1.33459
0.9950	3.40	2.72	18.7	1.33463

[1] This table gives alcohol by volume and by weight per cent. at 15.56° C., in terms of the specific gravity, apparent, at 20° C., and in terms of immersion refractometer readings at 20° C. The table was compiled by the author from those of Gilpin, Drinkwater and Squibb, U. S. Dept. Agr. Bur. Chem. Bull. No. 65 (1902), St. John based on that of Doroschevski and Dvorzhanchik, *J. Russ. Phys. Chem. Soc.* 40, 101 (1908) U. S. Bur. Standards Circ. No. 19 (1924). The various relationships are given to the nearest 0.1 per cent. alcohol by volume.

TABLE 4. ETHYL ALCOHOL—*Continued*

Apparent specific gravity 20°C/20°C.	Alcohol by volume per cent	Alcohol by weight per cent	Scale reading immersion refractometer at 20°C.	Index of refraction 20°C.
0.9949	3.50	2.80	18.8	1.33466
0.9948	3.60	2.88	19.0	1.33474
0.9946	3.70	2.96	19.2	1.33482
0.9945	3.80	3.04	19.3	1.33486
0.9943	3.90	3.12	19.4	1.33489
0.9942	4.00	3.20	19.6	1.33497
0.9941	4.10	3.28	19.7	1.33501
0.9939	4.20	3.36	19.8	1.33505
0.9938	4.30	3.44	20.0	1.33513
0.9936	4.40	3.52	20.1	1.33517
0.9935	4.50	3.60	20.2	1.33520
0.9934	4.60	3.68	20.4	1.33528
0.9932	4.70	3.76	20.5	1.33532
0.9931	4.80	3.84	20.6	1.33536
0.9930	4.90	3.92	20.8	1.33543
0.9928	5.00	4.00	21.0	1.33551
0.9927	5.10	4.08	21.1	1.33555
0.9926	5.20	4.16	21.2	1.33559
0.9924	5.30	4.24	21.4	1.33566
0.9923	5.40	4.32	21.5	1.33570
0.9922	5.50	4.40	21.6	1.33574
0.9920	5.60	4.48	21.8	1.33582
0.9919	5.70	4.56	21.9	1.33586
0.9917	5.80	4.64	22.0	1.33590
0.9916	5.90	4.72	22.2	1.33597
0.9915	6.00	4.80	22.4	1.33605
0.9914	6.10	4.88	22.5	1.33609
0.9912	6.20	4.96	22.6	1.33613
0.9911	6.30	5.05	22.8	1.33620
0.9910	6.40	5.13	22.9	1.33624
0.9908	6.50	5.21	23.0	1.33628
0.9907	6.60	5.29	23.2	1.33636
0.9906	6.70	5.37	23.3	1.33640
0.9905	6.80	5.45	23.4	1.33643
0.9903	6.90	5.53	23.6	1.33651
0.9902	7.00	5.61	23.8	1.33659
0.9901	7.10	5.69	23.9	1.33663
0.9899	7.20	5.77	24.0	1.33666
0.9898	7.30	5.86	24.2	1.33674
0.9897	7.40	5.94	24.3	1.33678
0.9896	7.50	6.02	24.4	1.33682
0.9894	7.60	6.10	24.6	1.33689
0.9893	7.70	6.18	24.8	1.33697
0.9892	7.80	6.26	24.9	1.33701
0.9891	7.90	6.34	25.0	1.33705

TABLE 4. ETHYL ALCOHOL—*Continued*

Apparent specific gravity 20°C./20°C.	Alcohol by volume per cent	Alcohol by weight per cent	Scale reading immersion refractometer at 20°C.	Index of refraction 20°C.
0.9889	8.00	6.42	25.2	1.33712
0.9888	8.10	6.50	25.4	1.33720
0.9887	8.20	6.58	25.5	1.33724
0.9886	8.30	6.67	25.6	1.33728
0.9885	8.40	6.75	25.8	1.33735
0.9883	8.50	6.83	25.9	1.33739
0.9882	8.60	6.91	26.0	1.33743
0.9881	8.70	6.99	26.2	1.33751
0.9879	8.80	7.07	26.4	1.33758
0.9878	8.90	7.15	26.5	1.33762
0.9877	9.00	7.23	26.6	1.33766
0.9876	9.10	7.31	26.8	1.33774
0.9875	9.20	7.39	27.0	1.33781
0.9873	9.30	7.48	27.1	1.33785
0.9872	9.40	7.56	27.2	1.33789
0.9871	9.50	7.64	27.4	1.33796
0.9870	9.60	7.72	27.5	1.33800
0.9869	9.70	7.80	27.7	1.33808
0.9867	9.80	7.88	27.8	1.33812
0.9866	9.90	7.96	28.0	1.33820
0.9865	10.00	8.04	28.1	1.33824
0.9864	10.10	8.12	28.3	1.33831
0.9863	10.20	8.20	28.4	1.33835
0.9861	10.30	8.29	28.6	1.33842
0.9860	10.40	8.37	28.7	1.33846
0.9859	10.50	8.45	28.9	1.33854
0.9858	10.60	8.53	29.0	1.33858
0.9857	10.70	8.61	29.2	1.33865
0.9855	10.80	8.70	29.3	1.33869
0.9854	10.90	8.78	29.4	1.33873
0.9853	11.00	8.86	29.6	1.33881
0.9852	11.10	8.94	29.8	1.33888
0.9851	11.20	9.02	29.9	1.33892
0.9849	11.30	9.11	30.1	1.33900
0.9848	11.40	9.19	30.2	1.33904
0.9847	11.50	9.27	30.4	1.33911
0.9846	11.60	9.35	30.6	1.33919
0.9845	11.70	9.43	30.7	1.33922
0.9844	11.80	9.51	30.8	1.33926
0.9842	11.90	9.59	31.0	1.33934
0.9841	12.00	9.67	31.2	1.33942
0.9840	12.10	9.75	31.3	1.33946
0.9839	12.20	9.83	31.4	1.33949
0.9838	12.30	9.92	31.6	1.33957
0.9837	12.40	10.00	31.8	1.33964

TABLE 4. ETHYL ALCOHOL—*Continued*

Apparent specific gravity 20°C./20°C.	Alcohol by volume per cent	Alcohol by weight per cent	Scale reading immersion refractometer at 20°C.	Index of refraction 20°C.
0.9835	12.50	10.08	31.9	1.33968
0.9834	12.60	10.16	32.0	1.33972
0.9833	12.70	10.24	32.2	1.33980
0.9832	12.80	10.33	32.4	1.33987
0.9831	12.90	10.41	32.5	1.33991
0.9830	13.00	10.49	32.6	1.33995
0.9829	13.10	10.57	32.8	1.34002
0.9827	13.20	10.65	33.0	1.34010
0.9826	13.30	10.74	33.1	1.34014
0.9825	13.40	10.82	33.3	1.34021
0.9824	13.50	10.90	33.4	1.34025
0.9823	13.60	10.98	33.6	1.34033
0.9822	13.70	11.06	33.8	1.34040
0.9820	13.80	11.15	33.9	1.34044
0.9819	13.90	11.23	34.1	1.34052
0.9818	14.00	11.31	34.2	1.34056
0.9817	14.10	11.39	34.4	1.34063
0.9816	14.20	11.47	34.5	1.34067
0.9815	14.30	11.56	34.7	1.34074
0.9814	14.40	11.64	34.8	1.34078
0.9813	14.50	11.72	35.0	1.34086
0.9812	14.60	11.80	35.2	1.34094
0.9810	14.70	11.88	35.3	1.34098
0.9809	14.80	11.97	35.5	1.34105
0.9808	14.90	12.05	35.6	1.34109
0.9807	15.00	12.13	35.8	1.34116
0.9806	15.10	12.21	36.0	1.34124
0.9805	15.20	12.29	36.1	1.34128
0.9804	15.30	12.38	36.3	1.34135
0.9803	15.40	12.46	36.4	1.34139
0.9801	15.50	12.54	36.6	1.34146
0.9800	15.60	12.62	36.8	1.34154
0.9799	15.70	12.70	36.9	1.34158
0.9798	15.80	12.79	37.1	1.34165
0.9797	15.90	12.87	37.2	1.34169
0.9796	16.00	12.95	37.4	1.34177
0.9795	16.10	13.03	37.5	1.34181
0.9794	16.20	13.12	37.7	1.34188
0.9793	16.30	13.20	37.9	1.34195
0.9792	16.40	13.29	38.0	1.34199
0.9791	16.50	13.37	38.2	1.34207
0.9789	16.60	13.45	38.3	1.34211
0.9788	16.70	13.53	38.5	1.34218
0.9787	16.80	13.62	38.7	1.34226
0.9786	16.90	13.70	38.8	1.34230

TABLE 4. ETHYL ALCOHOL—*Continued*

Apparent specific gravity 20°C./20°C.	Alcohol by volume per cent	Alcohol by weight per cent	Scale reading immersion refractometer at 20°C.	Index of refraction 20°C.
0.9785	17.00	13.78	39.0	1.34237
0.9784	17.10	13.86	39.1	1.34241
0.9783	17.20	13.94	39.3	1.34248
0.9782	17.30	14.03	39.5	1.34256
0.9781	17.40	14.11	39.6	1.34260
0.9780	17.50	14.19	39.8	1.34267
0.9779	17.60	14.27	40.0	1.34275
0.9778	17.70	14.35	40.1	1.34279
0.9776	17.80	14.44	40.3	1.34286
0.9775	17.90	14.52	40.4	1.34290
0.9774	18.00	14.60	40.6	1.34298
0.9773	18.10	14.68	40.8	1.34305
0.9772	18.20	14.77	40.9	1.34309
0.9771	18.30	14.85	41.1	1.34316
0.9770	18.40	14.94	41.2	1.34320
0.9769	18.50	15.02	41.4	1.34328
0.9768	18.60	15.10	41.6	1.34335
0.9767	18.70	15.18	41.7	1.34339
0.9766	18.80	15.27	41.9	1.34346
0.9765	18.90	15.38	42.0	1.34350
0.9764	19.00	15.43	42.2	1.34358
0.9763	19.10	15.51	42.4	1.34365
0.9762	19.20	15.59	42.6	1.34373
0.9761	19.30	15.68	42.7	1.34377
0.9760	19.40	15.76	42.9	1.34384
0.9758	19.50	15.84	43.0	1.34388
0.9757	19.60	15.93	43.2	1.34395
0.9756	19.70	16.01	43.4	1.34403
0.9755	19.80	16.09	43.5	1.34407
0.9754	19.90	16.18	43.7	1.34414
0.9753	20.00	16.26	43.8	1.34418
0.9752	20.10	16.34	44.0	1.34426
0.9751	20.20	16.42	44.2	1.34433
0.9750	20.30	16.51	44.4	1.34440
0.9749	20.40	16.59	44.5	1.34444
0.9748	20.50	16.67	44.7	1.34452
0.9747	20.60	16.75	44.8	1.34456
0.9746	20.70	16.84	45.0	1.34463
0.9745	20.80	16.92	45.2	1.34470
0.9744	20.90	17.01	45.3	1.34474
0.9742	21.00	17.09	45.5	1.34482
0.9741	21.10	17.17	45.7	1.34489
0.9740	21.20	17.26	45.8	1.34493
0.9739	21.30	17.34	46.0	1.34500
0.9738	21.40	17.43	46.2	1.34508

TABLE 4. ETHYL ALCOHOL—*Continued*

Apparent specific gravity 20°C./20°C.	Alcohol by volume per cent	Alcohol by weight per cent	Scale reading immersion refractometer at 20°C.	Index of refraction 20°C.
0.9737	21.50	17.51	46.3	1.34512
0.9736	21.60	17.59	46.5	1.34520
0.9735	21.70	17.67	46.7	1.34526
0.9734	21.80	17.76	46.8	1.34530
0.9733	21.90	17.84	47.0	1.34538
0.9732	22.00	17.92	47.2	1.34545
0.9731	22.10	18.00	47.3	1.34549
0.9730	22.20	18.09	47.5	1.34556
0.9729	22.30	18.17	47.6	1.34560
0.9727	22.40	18.26	47.8	1.34568
0.9726	22.50	18.34	48.0	1.34575
0.9725	22.60	18.42	48.2	1.34583
0.9724	22.70	18.51	48.3	1.34587
0.9723	22.80	18.59	48.5	1.34594
0.9722	22.90	18.68	48.6	1.34598
0.9721	23.00	18.76	48.8	1.34605
0.9720	23.10	18.84	49.0	1.34613
0.9719	23.20	18.92	49.1	1.34617
0.9717	23.30	19.01	49.3	1.34624
0.9716	23.40	19.09	49.5	1.34631
0.9715	23.50	19.17	49.6	1.34635
0.9714	23.60	19.25	49.8	1.34643
0.9713	23.70	19.34	50.0	1.34650
0.9712	23.80	19.42	50.1	1.34654
0.9711	23.90	19.51	50.3	1.34661
0.9710	24.00	19.59	50.4	1.34665
0.9708	24.10	19.67	50.6	1.34672
0.9707	24.20	19.76	50.8	1.34680
0.9706	24.30	19.84	50.9	1.34684
0.9705	24.40	19.93	51.1	1.34691
0.9704	24.50	20.01	51.2	1.34695
0.9703	24.60	20.09	51.4	1.34702
0.9702	24.70	20.18	51.6	1.34710
0.9701	24.80	20.26	51.7	1.34714
0.9699	24.90	20.35	51.9	1.34721
0.9698	25.00	20.43	52.1	1.34728
0.9697	25.10	20.51	52.2	1.34732
0.9696	25.20	20.60	52.4	1.34740
0.9695	25.30	20.68	52.5	1.34744
0.9694	25.40	20.77	52.7	1.34751
0.9692	25.50	20.85	52.9	1.34758
0.9691	25.60	20.93	53.0	1.34762
0.9690	25.70	21.02	53.2	1.34769
0.9689	25.80	21.10	53.3	1.34773
0.9688	25.90	21.19	53.5	1.34780

TABLE 4. ETHYL ALCOHOL—*Continued*

Apparent specific gravity 20°C./20°C.	Alcohol by volume per cent	Alcohol by weight per cent	Scale reading immersion refractometer at 20°C.	Index of refraction 20°C.
0.9687	26.00	21.27	53.7	1.34788
0.9686	26.10	21.35	53.8	1.34792
0.9684	26.20	21.44	54.0	1.34799
0.9683	26.30	21.52	54.1	1.34803
0.9682	26.40	21.61	54.3	1.34810
0.9681	26.50	21.69	54.4	1.34814
0.9680	26.60	21.77	54.6	1.34821
0.9679	26.70	21.86	54.8	1.34829
0.9677	26.80	21.94	54.9	1.34833
0.9676	26.90	22.03	55.1	1.34840
0.9675	27.00	22.11	55.2	1.34844
0.9674	27.10	22.20	55.4	1.34851
0.9673	27.20	22.28	55.6	1.34858
0.9672	27.30	22.37	55.7	1.34862
0.9670	27.40	22.45	55.9	1.34869
0.9669	27.50	22.54	56.0	1.34873
0.9668	27.60	22.62	56.2	1.34880
0.9667	27.70	22.71	56.4	1.34888
0.9665	27.80	22.79	56.5	1.34892
0.9664	27.90	22.88	56.7	1.34899
0.9663	28.00	22.96	56.8	1.34903
0.9662	28.10	23.04	57.0	1.34910
0.9661	28.20	23.13	57.2	1.34918
0.9660	28.30	23.21	57.3	1.34922
0.9659	28.40	23.30	57.5	1.34928
0.9657	28.50	23.38	57.6	1.34932
0.9656	28.60	23.47	57.8	1.34940
0.9655	28.70	23.55	58.0	1.34947
0.9654	28.80	23.64	58.1	1.34951
0.9653	28.90	23.72	58.3	1.34958
0.9651	29.00	23.81	58.4	1.34962
0.9650	29.10	23.89	58.6	1.34969
0.9649	29.20	23.98	58.8	1.34977
0.9648	29.30	24.06	58.9	1.34980
0.9647	29.40	24.15	59.1	1.34987
0.9645	29.50	24.23	59.2	1.34991
0.9644	29.60	24.32	59.4	1.34999
0.9643	29.70	24.40	59.5	1.35003
0.9642	29.80	24.49	59.7	1.35010
0.9640	29.90	24.57	59.8	1.35014
0.9639	30.00	24.66	60.0	1.35021
0.9638	30.10	24.74	60.2	1.35028
0.9637	30.20	24.83	60.3	1.35032
0.9635	30.30	24.91	60.5	1.35039
0.9634	30.40	25.00	60.6	1.35043

TABLE 4. ETHYL ALCOHOL—*Continued*

Apparent specific gravity 20°C./20°C.	Alcohol by volume per cent	Alcohol by weight per cent	Scale reading immersion refractometer at 20°C.	Index of refraction 20°C.
0.9633	30.50	25.08	60.8	1.35050
0.9632	30.60	25.17	60.9	1.35054
0.9630	30.70	25.25	61.1	1.35061
0.9629	30.80	25.34	61.2	1.35065
0.9628	30.90	25.42	61.4	1.35073
0.9627	31.00	25.51	61.6	1.35080
0.9626	31.10	25.60	61.7	1.35084
0.9624	31.20	25.68	61.9	1.35091
0.9623	31.30	25.77	62.0	1.35095
0.9622	31.40	25.85	62.2	1.35102
0.9620	31.50	25.94	62.3	1.35106
0.9619	31.60	26.03	62.5	1.35113
0.9618	31.70	26.11	62.6	1.35117
0.9616	31.80	26.20	62.8	1.35124
0.9615	31.90	26.28	62.9	1.35128
0.9614	32.00	26.37	63.1	1.35135
0.9612	32.10	26.46	63.2	1.35139
0.9611	32.20	26.54	63.4	1.35146
0.9610	32.30	26.63	63.5	1.35150
0.9608	32.40	26.71	63.6	1.35154
0.9607	32.50	26.80	63.8	1.35161
0.9606	32.60	26.89	63.9	1.35165
0.9604	32.70	26.97	64.1	1.35172
0.9603	32.80	27.06	64.2	1.35176
0.9602	32.90	27.14	64.4	1.35183
0.9600	33.00	27.23	64.5	1.35187
0.9599	33.10	27.32	64.7	1.35194
0.9598	33.20	27.40	64.8	1.35198
0.9596	33.30	27.49	64.9	1.35202
0.9595	33.40	27.57	65.1	1.35209
0.9594	33.50	27.66	65.2	1.35212
0.9592	33.60	27.75	65.4	1.35220
0.9591	33.70	27.83	65.5	1.35224
0.9590	33.80	27.92	65.7	1.35230
0.9588	33.90	28.00	65.8	1.35234
0.9587	34.00	28.09	65.9	1.35238
0.9585	34.10	28.18	66.1	1.35245
0.9584	34.20	28.26	66.2	1.35249
0.9583	34.30	28.35	66.4	1.35256
0.9581	34.40	28.43	66.5	1.35260
0.9580	34.50	28.52	66.6	1.35264
0.9578	34.60	28.61	66.8	1.35271
0.9577	34.70	28.70	66.9	1.35275
0.9576	34.80	28.78	67.1	1.35282
0.9574	34.90	28.87	67.2	1.35286

TABLE 4. ETHYL ALCOHOL—*Continued*

Apparent specific gravity 20°C./20°C.	Alcohol by volume per cent	Alcohol by weight per cent	Scale reading immersion refractometer at 20°C.	Index of refraction 20°C.
0.9573	35.00	28.96	67.3	1.35290
0.9571	35.10	29.05	67.5	1.35296
0.9570	35.20	29.13	67.6	1.35300
0.9568	35.30	29.22	67.7	1.35304
0.9567	35.40	29.30	67.9	1.35311
0.9566	35.50	29.38	68.0	1.35315
0.9564	35.60	29.48	68.1	1.35319
0.9563	35.70	29.57	68.3	1.35325
0.9561	35.80	29.65	68.4	1.35329
0.9560	35.90	29.74	68.5	1.35331
0.9558	36.00	29.83	68.7	1.35340
0.9557	36.10	29.92	68.8	1.35344
0.9555	36.20	30.00	68.9	1.35348
0.9554	36.30	30.09	69.1	1.35354
0.9552	36.40	30.17	69.2	1.35359
0.9551	36.50	30.26	69.3	1.35363
0.9549	36.60	30.35	69.4	1.35366
0.9548	36.70	30.44	69.6	1.35373
0.9546	36.80	30.52	69.7	1.35377
0.9545	36.90	30.61	69.8	1.35381
0.9543	37.00	30.70	70.0	1.35388
0.9542	37.10	30.79	70.1	1.35392
0.9540	37.20	30.88	70.2	1.35395
0.9539	37.30	30.96	70.3	1.35399
0.9537	37.40	31.05	70.5	1.35406
0.9536	37.50	31.14	70.6	1.35410
0.9534	37.60	31.23	70.7	1.35414
0.9533	37.70	31.32	70.8	1.35417
0.9531	37.80	31.40	71.0	1.35424
0.9530	37.90	31.49	71.1	1.35428
0.9528	38.00	31.58	71.2	1.35432
0.9527	38.10	31.67	71.3	1.35436
0.9525	38.20	31.76	71.4	1.35440
0.9524	38.30	31.85	71.6	1.35446
0.9522	38.40	31.94	71.7	1.35450
0.9520	38.50	32.03	71.8	1.35454
0.9518	38.60	32.12	71.9	1.35459
0.9517	38.70	32.20	72.1	1.35465
0.9516	38.80	32.29	72.2	1.35468
0.9514	38.90	32.37	72.3	1.35472
0.9513	39.00	32.46	72.4	1.35475
0.9511	39.10	32.55	72.5	1.35479
0.9509	39.20	32.64	72.6	1.35483
0.9508	39.30	32.72	72.8	1.35490
0.9506	39.40	32.81	72.9	1.35494

TABLE 4. ETHYL ALCOHOL—*Continued*

Apparent specific gravity 20°C. 20°C.	Alcohol by volume per cent	Alcohol by weight per cent	Scale reading immersion refractometer at 20°C.	Index of refraction 20°C.
0.9505	39.50	32.90	73.0	1.35497
0.9503	39.60	32.99	73.1	1.35501
0.9501	39.70	33.08	73.2	1.35504
0.9500	39.80	33.17	73.3	1.35508
0.9498	39.90	33.27	73.4	1.35512
0.9497	40.00	33.35	73.6	1.35519
0.9495	40.10	33.44	73.7	1.35523
0.9493	40.20	33.53	73.8	1.35526
0.9492	40.30	33.61	73.9	1.35530
0.9490	40.40	33.70	74.0	1.35533
0.9489	40.50	33.79	73.2	1.35541
0.9487	40.60	33.88	74.3	1.35545
0.9485	40.70	33.97	74.4	1.35548
0.9484	40.80	34.06	74.5	1.35552
0.9482	40.90	34.15	74.6	1.35555
0.9480	41.00	34.24	74.7	1.35559
0.9479	41.10	34.33	74.8	1.35563
0.9477	41.20	34.42	75.0	1.35570
0.9475	41.30	34.50	75.1	1.35574
0.9474	41.40	34.59	75.2	1.35577
0.9472	41.50	34.68	75.3	1.35581
0.9470	41.60	34.77	75.4	1.35584
0.9469	41.70	34.86	75.5	1.35588
0.9467	41.80	34.95	75.6	1.35592
0.9465	41.90	35.04	75.8	1.35599
0.9464	42.00	35.13	75.9	1.35603
0.9462	42.10	35.22	76.0	1.35606
0.9460	42.20	35.31	76.1	1.35610
0.9459	42.30	35.40	76.2	1.35613
0.9457	42.40	35.49	76.3	1.35617
0.9455	42.50	35.58	76.4	1.35621
0.9454	42.60	35.67	76.5	1.35625
0.9452	42.70	35.76	76.6	1.35628
0.9450	42.80	35.85	76.8	1.35635
0.9448	42.90	35.94	76.9	1.35639
0.9447	43.00	36.03	77.0	1.35642
0.9445	43.10	36.12	77.1	1.35646
0.9443	43.20	36.21	77.2	1.35650
0.9442	43.30	36.30	77.3	1.35654
0.9440	43.40	36.39	77.4	1.35657
0.9438	43.50	36.48	77.5	1.35661
0.9436	43.60	36.57	77.6	1.35664
0.9435	43.70	36.66	77.7	1.35668
0.9433	43.80	36.75	77.8	1.35671
0.9431	43.90	36.84	78.0	1.35678

TABLE 4. ETHYL ALCOHOL—*Continued*

Apparent specific gravity 20°C./20°C.	Alcohol by volume per cent	Alcohol by weight per cent	Scale reading immersion refractometer at 20°C.	Index of refraction 20°C.
0.9429	44.00	36.93	78.1	1.35682
0.9428	44.10	37.02	78.2	1.35686
0.9426	44.20	37.11	78.3	1.35690
0.9424	44.30	37.21	78.4	1.35693
0.9422	44.40	37.30	78.5	1.35697
0.9420	44.50	37.39	78.6	1.35700
0.9419	44.60	37.48	78.7	1.35704
0.9417	44.70	37.57	78.8	1.35707
0.9415	44.80	37.66	78.9	1.35711
0.9413	44.90	37.76	79.0	1.35715
0.9412	45.00	37.84	79.1	1.35719
0.9410	45.10	37.93	79.2	1.35722
0.9408	45.20	38.02	79.3	1.35726
0.9406	45.30	38.12	79.4	1.35729
0.9404	45.40	38.21	79.5	1.35733
0.9402	45.50	38.30	79.6	1.35736
0.9401	45.60	38.39	79.7	1.35740
0.9399	45.70	38.48	79.8	1.35744
0.9397	45.80	38.57	79.9	1.35748
0.9395	45.90	38.66	80.0	1.35751
0.9393	46.00	38.75		
0.9384	46.50	39.21		
0.9375	47.00	39.67		
0.9365	47.50	40.13		
0.9356	48.00	40.60		
0.9345	48.50	41.06		
0.9337	49.00	41.52		
0.9327	49.50	41.99		
0.9317	50.00	42.47		

SUBJECT INDEX

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